

NEW MEMBRANE TECHNOLOGIES - NANOTUBE MEMBRANES FOR
BIOTECHNOLOGICAL APPLICATIONS AND POLYANILINE FILMS FOR
CORROSION INHIBITION

By

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This dissertation is dedicated to my parents, Marica and Zeljko Gasparac.

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Abstract of Dissertation Presented to the Graduate School
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By

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December 2003

Chair: John R. Reynolds
Major Department: Chemistry

Chapter 1 provides background information on the template synthesis of nanomaterials. A description of the types of membranes used for template synthesis is given. Different chemistries that have been used to prepare template-synthesized structures are provided. Template synthesis has been used to prepare some important nanostructures. Polycarbonate membranes were used to prepare gold nanotubes using electroless gold template method. SiO₂ nano-test-tubes were made by dipping the alumina template membrane in the sol and heating. An introduction to conductive is presented here as well.

Chapter 2 describes the transport of DNA molecules through nanopore membranes. We are interested in how pore diameter of the membrane affects rate and selectivity of DNA transport of different size and charge. Commercially available microporous polycarbonate membrane filters were used. DNA chains can be driven through the nanopore via the electrokinetic transport processes of electrophoresis and electroosmotic

flow, as well as by diffusion. To our knowledge, there have been no quantitative studies of the relative importance of the electrokinetic and diffusive components for DNA transport in a nanopore system.

Chapter 3 describes a sol-gel template synthesis process that is used to produce silica nano-test-tubes within the pores of alumina templates. These silica nano-test-tubes are important because of the ease with which nearly any desired chemical or biochemical reagent can be covalently attached to their inside and outside surfaces. Inner and outer surfaces of the silica nano-test-tubes were functionalized using well-known silane chemistry. Green fluorescent silane was attached to the inner surfaces. The outer SiO_2 nano-test-tube surfaces were antibody functionalized using aldehyde methoxysilane linker. One of our key long-range objective is to develop nanotube technology for delivering biomolecules (e.g., DNA) to living cells.

Chapter 4 focuses on the mechanism by which polyaniline (PANI) films passivate stainless steel surfaces in highly corrosive H_2SO_4 solution. A variety of experimental methods including measurements of the open circuit potential, Auger depth profiling, and the scanning reference electrode technique (SRET) was used. These studies have shown that passivation is achieved because the oxidized and protically-doped emeraldine-salt form of PANI holds the potential of the underlying stainless steel electrode in the passive region. Because of this electrostatic mechanism of corrosion inhibition, the entire stainless steel surface does not have to be coated with PANI in order to achieve passivation.

CHAPTER 1 INTRODUCTION TO TEMPLATE SYNTHESIS

Introduction

Nanoscale science and technology is an emerging subdiscipline of the chemical and materials sciences that deals with the development of methods for synthesizing nanoscopic particles of a desired material and with scientific investigations of the nanomaterial obtained (1). Nanomaterials have numerous possible commercial and technological applications including use in electronic and optical devices, drug delivery etc (2). Nanostructures have received growing interests due to their fascinating properties which for certain applications can be superior to their bulk counterparts (3-6). The engineer's "top down" approach is to carve out nanometer-scale objects from a substrate lithographically and the chemist's "bottom up" approach is to assemble them from molecular-scale precursors (7).

In this chapter the "bottom up" approach was used to prepare one-dimensional (1D) nanotubes and nano-test-tubes. An overview of synthetic strategies that have been exploited to achieve 1D growth is given. Template synthesis represents a straightforward route to 1D nanostructures. The template simply serves as a scaffold within which a different material is generated in situ and shaped into a nanostructure with its morphology complementary to that of the template (3). 1D nanostructures have been synthesized using chemical methods. The anisotropic growth of 1D nanostructures is directed or confined by the template.

A number of different nanopore materials have been used for template synthesis. For example, the Martin group and, several other groups have used polycarbonate membranes to prepare nanostructures composed of conductive polymers (8-14), metals (15-19), semiconductors (20), single-crystalline and polycrystalline nanowires (21, 22). Some of these nanowires approach molecular dimensions that might be useful as mimics in biological science and technology (23). Most nanowires synthesized using template methods are polycrystalline in nature.

Another natural 1D template material is nanopore alumina. When grown under appropriate conditions, cylindrical pores form normal to the surface of the membrane (*vide infra*, Figure 1-6). The Martin group has used porous alumina as a template material to prepare polymers (24), lithium ion battery materials (25), carbon (26) and semiconductors (27). Other groups have used alumina to prepare metallic barcodes (28) and nanorods (29, 30).

Background

Template Synthesis

Many methods have been developed for the fabrication of nanoparticles in recent years. Our group has pioneered the method called template synthesis for the preparation of a variety of nanomaterials (31). This process involves synthesizing a desired material within the pores of a nanopore membrane. This method has several useful features. First, this method is extremely general with regard to the types of materials that can be prepared. Second, depending on the properties of the materials and the chemistry on the pore wall the nanotubes (hollow) and nanowires (solid) with very small diameters can be prepared. The Martin group has prepared nanotubes and nanowires composed of

conductive polymers (13, 32, 33), metals (16, 34, 35), semiconductors (36) and carbon (26, 37).

In particular, Martin et al. have investigated nanowires and nanotubes composed of the conductive polymers polypyrrole, poly(3-methylthiophene) and polyaniline. These materials were prepared by oxidative polymerization of the corresponding monomer (38). Oxidative polymerization was achieved either electrochemically (14, 32) or chemically (11, 23, 39). Chemical template synthesis using chemical oxidizing agent was used by other groups as well (40-42). Martin et al. showed that polypyrrole nanotubes can be filled with enzymes to make a high surface area nanoreactor (38). This technology might have significant application in drug delivery and microelectronics (43).

Nanoscale metals have interesting optical (20, 44) and electronic properties (45). For example, colloidal suspensions of gold can be red, purple, or blue, depending on the size of the spherical gold particles (38, 46). Metals can also be deposited within the pores of the template membranes by either electrochemical or chemical ("electroless") reduction of the appropriate metal ion (38). The Martin research group has electrolessly plated gold within the pores of polycarbonate template membranes (Figure 1-2).

They have shown that because the inside diameters of these nanotubes can be small relative to molecular dimensions, the nanotube membrane can be used to cleanly separate small molecules on the basis of molecular size (47). For example, they have shown that a membrane with nanotubes having an inside diameter of 0.6 nm will transport pyridine (MW = 79) but will not transport quinine (MW = 324) (47). Second, they have shown that in addition to this size-based selectivity, chemical transport selectivity can be introduced into these membranes (48, 49). Third, they have shown that gold nanotube

membranes show selective ion transport due to excess charge density that can be present on the inner walls of the tubes. The tubes reject ions of the same sign, and transport ions of the opposite sign, as this excess charge.

The Martin group has shown that biopolymers such as proteins can be separated using these membranes (50, 51, 52). The effect of nanotube inside diameter on rate and selectivity of protein transport was investigated for proteins lysozyme (Lys), bovine serum albumin (BSA), and β -lactoglobulin A (50). Transport selectivity increased as the inside diameter of the nanotubes decreased. Mitchell et al. developed the concept of smart nanotubes that can be used for bioseparation and biocatalysis (53). For example, they have shown that nanotubes containing an enantioselective antibody selectively extract the enantiomer of a drug molecule that binds to the antibody (53).

Recently, Gasparac and Martin have focused on DNA transport studies within bare (54) and gold plated polycarbonate membranes. The single-stranded homo-oligonucleotides made of thymidine bases (poly(T)_x), $x = 8, 24, 32$ and 64 were driven through polycarbonate membrane by diffusion and electrophoretically. It has been shown that diffusive flux decreases with increasing size (base number) of the poly(T)_x . Thus, these membranes act as size-based ultrafiltration membranes for the poly(T)_x 's.

Kressin et al. have shown that II-VI semiconductor films can be prepared electrochemically (55). Klein et al used alumina template membranes to deposit nickel into the membrane (36).

Finally, these hollow and solid nanostructures can be assembled into a variety of architectures. They can remain inside the pores or can be freed from the template membrane and collected as an ensemble of free nanoparticles (31).

Polycarbonate Template Membranes

Polycarbonate is a clear plastic used to make shatterproof windows and lightweight eyeglass lenses. It gets its name from the carbonate groups in its backbone chain (Figure 1-1). Polycarbonate is made from bisphenol A and phosgene. Polycarbonate membranes are negatively charged and can be hydrophobic or hydrophilic when wetted with the wetting agent polyvinylpyrrolidone (PVP). These membranes offer distinct advantages over conventional membranes due to their superior strength, negligible adsorption of filtrate, excellent chemical resistance and thermal stability, and biological inertness (56, 57). Their pore size, shape and density can be varied in a controllable manner so that a membrane with the required transport and retention characteristics can be produced.

A number of companies (e.g. Nucleopore and Osmonics Poretics) sell micro- and nanoporous polymeric filtration membranes that have been prepared via the "track-etch" method (58). These membranes contain cylindrical pores of uniform diameter (Figure 1-1). The pores are randomly distributed across the membrane surface. Membranes with a wide range of pore diameters (down to 10 nm and as large as $\sim 10\ \mu\text{m}$) and pore densities, approaching $10^9\ \text{pores}/\text{cm}^2$, are available commercially.

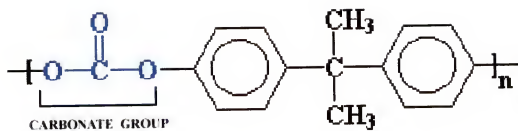


Figure 1-1. Structure of polycarbonate material.

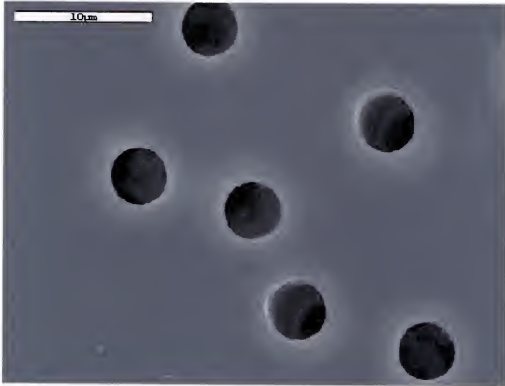


Figure 1-2. Scanning electron micrograph (SEM) of the surface of a microporous polycarbonate membrane prepared via the “track-etch” method. Pores with 5 μm in diameter are shown.

Track-etch technique

In the early 1960s, Price and Walker discovered that damage tracks produced in mica by high-energy particles could be preferentially etched to yield pores with diameters dependent on the etching time (59, 60). The procedure for etching damage tracks was subsequently perfected for other minerals and plastics (60). The most commonly used materials to prepare track-etch membranes of this type are polycarbonate and polyester; however, a number of other materials are also amenable to the track-etch process. The track-etch process entails bombarding a solid material ($\sim 10\ \mu\text{m}$ -thick polycarbonate film in this case) with a collimated beam of high-energy nuclear fission fragments to create parallel damage tracks in the film (61).

There are two basic methods of producing tracks in the foils to be transformed into porous membranes. The first method is based on the irradiation with fragments from the fission of heavy nuclei such as californium or uranium (60, 62). The advantages of the fission fragment tracking are with high porosity membranes, good stability of a particle flux and relatively low cost. However, the contamination of the tracked foil with radioactive products is produced and is the big disadvantage of using this method. The second method is based on the use of ion beams from accelerators (60, 63, 64). The advantages of the accelerator tracking method are no radioactive contamination of the material and particles heavier than fission fragments can be used. The cost of irradiation is usually high.

The damage tracks are then etched into monodisperse cylindrical pores by exposing the film to a concentrated solution of aqueous base (Figure 1-3) (65). During chemical etching the damaged zone of a track is removed and transformed into a hollow channel

(60). It is the pore-size determining and pore-shape determining stage of the technology.

The bulk etch rate depends on the material, on the etchant composition and on the temperature. The diameter of the pores is determined by the etch time and the etch-solution temperature (66, 67). The density of pores is determined by exposure time to the fission-fragment beam.

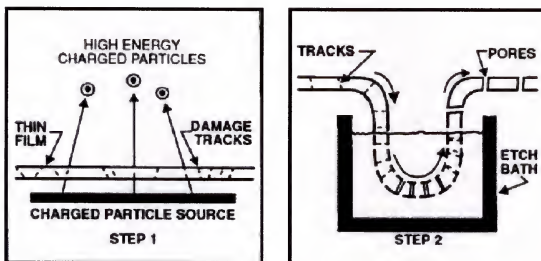


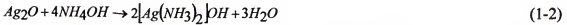
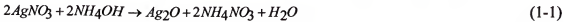
Figure 1-3. Scheme of track etch process. Image obtained from GE Osmonics Labstore, <http://www.osmolabstore.com/scripts/OsmoLabPage.dll?BuildPage&1&1&1070>, 11/04/2003

Electroless gold template method

The Martin research group has pioneered a new class of membranes that have unique transport properties (34, 47-49, 67-69). These membranes contain a collection of parallel monodisperse nanotubes, with inside diameters that can be as small as molecular dimensions (< 1 nm). The tubes are synthesized within the pores of the track-etched membrane filters discussed above via the template synthesis method.

Electroless metal deposition involves the use of a chemical reducing agent to plate a metal from solution onto a surface. The key requirement is to arrange the chemistry such that the kinetics of homogeneous electron transfer from the reducing agent to the

metal ion are slow (70). The method developed for electroless plating of the Au nanotubes can be summarized as follows. The template membrane is first “sensitized” by immersion into a SnCl_2 solution, which results in deposition of Sn^{II} onto all of the membrane’s surface (pore walls and membrane faces). The sensitized membrane is then immersed into an aqueous basic AgNO_3 solution. A complexing agent such as ammonia is added to the solution to prepare silver (I) complex (71). Equations 1-1 and 1-2 show the complex formation reactions and are thought to be as follows (72).



A surface redox reaction occurs (Equation 1-3) and Ag^{I} is reduced by Sn^{II} which yields adsorption of nanoscopic metallic Ag particles on the membrane surfaces.



The subscripts “surf” and “aq” denote species adsorbed to the membrane surfaces and species dissolved in solution, respectively. The membrane is then immersed into a commercial gold plating solution and a second surface redox reaction occurs, to yield Au nanoparticles on the surfaces (Equation 1-4).



These surface-bound Au nanoparticles are good autocatalysts for the reduction of Au^{I} to Au^0 using formaldehyde as the reducing agent. Concomitant oxidation of the silver to silver ions occurs. Reducing agents such as formalin, dextrose, hydrazine sulfate are commercially available and can be used as well. As a result, Au deposition begins at the pore walls, and Au nanotubes are obtained within the pores. In addition, the faces of the membrane also become coated with thin Au films (Figure 1-4). By controlling the

electroless plating time, the inside diameter of these nanotubes can be controlled at will, and inside diameters $< 1\text{ nm}$ can be obtained (48, 73) (Figure 1-5). The Martin group has shown that a variety of different materials can be deposited within these pores, and nanostructures with extraordinarily small diameters can be prepared (34, 47-49, 67-69).

Gas flux measurements

The inside diameters of the Au nanotubes plated within the template membranes can be approximated using a gas-permeation method (74). In general, gas transport in membranes can occur by four mechanisms: viscous flow, ultramicroporous sieving diffusion, solution-diffusion and Knudsen flow (75). Viscous flow occurs when the pore radius in the membrane is large relative to the mean-free path of the gas. Gases can be separated by ultramicrofiltration when the pore size is comparable to the diameter of the gas molecule. Solution-diffusion occurs when gas first dissolves in the membrane and then diffuses through the membrane. This occurs in membranes that do not contain pores.

Knudsen flow occurs when the average pore radius in the membrane is smaller than the mean-free path of the gas. The rate of Knudsen diffusion is described by the following equation (76, 77)

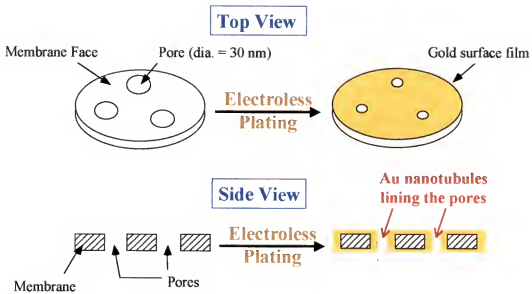


Figure 1-4. Scheme of electroless gold template method.

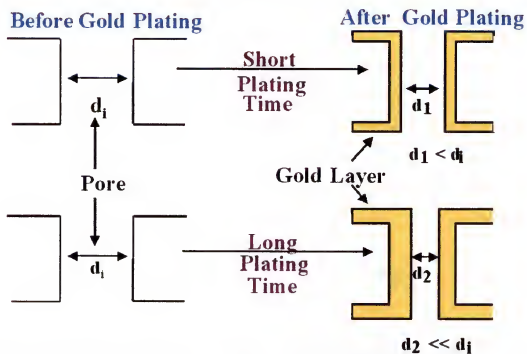


Figure 1-5. Side view of scheme showed in Figure 1-4.

$$Q = \frac{4}{3} \left[\frac{2\pi}{MR_0T} \right]^{1/2} \frac{nR^3P}{L} \quad (1-5)$$

where Q is the flux of gas (moles s^{-1}), n is number of pores, R is pore radius (cm), L is the membrane thickness (cm), P is the pressure difference across the membrane (dynes cm^{-2}), M is the molecular weight of the gas, R_0 is the gas constant (erg $K^{-1}mol^{-1}$), and T is the temperature (K).

I have used Equation 1-5 to calculate the nanotube diameter in Au nanotube membranes prepared for my studies. The gas transport cell has been described previously (78). After the Au nanotube membrane is placed between the upper and lower half-cells, both half-cells are evacuated. The upper half-cell is then pressurized with H_2 gas (34.7 psi). The change of the pressure (P) with time (t) in the lower half-cell is then monitored using a pressure transducer and a strip-chart recorder. The gas flux is determined from the linear portion of the P vs t transient (78). In our experiment we know all of the parameters in Equation 1-6 except R . Equation 1-5 assumes that the nanotubes have uniform inside diameters down their entire length and that the average pore radius in the membrane is smaller than the mean-free path of the gas.

Alumina Template Membranes

Ordered nanochannel-array materials have attracted increasing attention in recent years due to their utilization as templates for nanosize structures (79). For almost 50 years aluminum oxide (Al_2O_3) – alumina has been the subject of numerous investigations (80). Porous alumina membranes have been used extensively as a template (37, 81-84). Our group used alumina as template materials to prepare polymers (24), lithium ion battery materials (25), carbon (26) and semiconductors (27).

There are several features that make alumina membranes especially valuable as templates. They can be heated to high temperatures ($\sim 1000^{\circ}\text{C}$) without degradation and they are transparent in the visible region (70). Thus, it is possible to study the optical properties of the nanomaterial deposited within the alumina pore (84). The amphoteric property of alumina allows it to be dissolved away in acidic or basic solution to expose the nanostructures deposited within the pores.

Porous alumina membranes are prepared via the anodization of aluminum metal in acidic solution (85). The resulting pores are oriented perpendicular to the surface and are arranged in a regular lattice (86, 87). Pore densities of 10^9 to 10^{12} pores/cm² can be prepared (88). Pore size is dependent on the voltage used for oxidation and can be varied from ~ 10 nm to ~ 400 nm (89, 90). The pores are highly monodisperse and porosities can be greater than 50%. Typical membrane thickness can range from 10 to 100 μm . The thickness of the membrane is determined by the amount of charge transferred during aluminum oxidation. Alumina membranes are available commercially as a filtration membrane with pores of nominally 20, 100 and 200 nm diameters from Whatman International, Maidstone, England. Figure 1-6 shows a scanning electron micrograph of the surface and a cross section of such membrane. Our group prepared membranes of this type with pore diameters as large as 200 nm and as small as 5 nm (see Figure 4-1 in Chapter 4 for homegrown 50 nm alumina membrane).

In order to prepare alumina with highly monodisperse cylindrical pores, high-purity aluminum metal (99.999%) is used. Al is used as the anode and stainless steel as the cathode. Prior to Al anodization, high purity Al needs to be polished electrochemically in order to get a smooth surface to produce an even and flat alumina template. This process

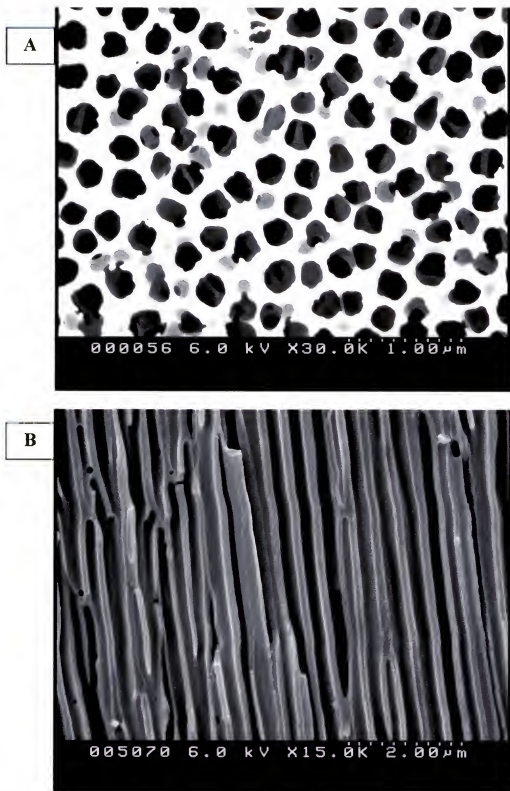


Figure 1-6. Scanning electron micrographs of alumina membrane. Top view (A) and side view of commercial 200 nm membrane (Whatman). Courtesy of Dr. Shifeng Hou.

is done in concentrated acid solution and at high temperatures to favor immediate dissolution of the alumina (87, 91). The electropolishing solution in our lab is 95% concentrated phosphoric acid, 5% concentrated sulfuric acid with 20 g/L CrO_3 added to prevent pitting. The electropolishing is done at 70° C and voltages between 10 and 15 V are applied for periods of 5 minutes. Two or three electropolishing steps will generally result in a mirror finish. Both electrodes (anode and cathode) are then immersed in aqueous acid electrolyte (sulfuric acid, phosphoric acid or oxalic acid) and voltage is supplied by a power supply. The size of the pores to be grown depends on the type of the acid that is used as an electrolyte (92).

Masuda et al. first developed a two-step process (93). They obtained pores with hexagonal packing and uniform pore size (94). In the first step an alumina film is grown for sufficient time to allow self-organization and homogenization of pore size (87, 91). This film is then dissolved in acid and pits or indentations are left in the underlying aluminum that corresponds to each pore. In the second step, the aluminum is then re-anodized using the same conditions as in the first step. The pores nucleate in the pits and continue to grow in a highly ordered and monodisperse fashion (93). Figure 1-7 shows a schematic diagram of a porous alumina oxide film grown on aluminum.

These porous alumina films can then be removed from the underlying aluminum and collected as a free-standing membrane. There are two methods of removing the alumina film. In the first method, the underlying aluminum metal is dissolved with HgCl_2 and the second process is called voltage reduction when the voltage is reduced to about 70% of its original value (95, 96). In the work described here, we are not going to

remove alumina film. We are going to use the alumina films that are still attached to the underlying aluminum.

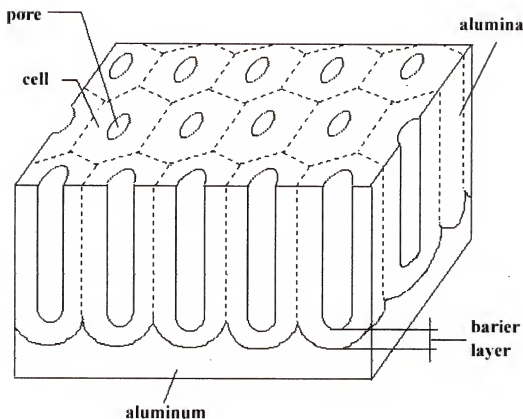


Figure 1-7. Scheme of a porous alumina oxide film grown on aluminum. Scheme obtained at <http://plaza.snu.ac.kr/~nanotube/image/AFM/template.jpg>

Sol-gel chemistry

Sol-gel technology was first discovered in the late 1800s and has been extensively studied since the early 1930s. Scientists tried to find a way to synthesize glass without using high temperatures to form glass. The high temperatures usually (1300 to 2000° C) destroy the crystallinity of the precursors needed for glass synthesis. A method using noncrystalline precursors that will produce high purity and high homogeneity materials while employing low temperatures was sought. In the 1970s monolithic (without cracks)

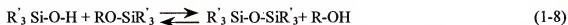
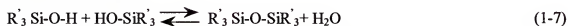
inorganic gels were formed at low temperatures and converted to glass without a high temperature melting process (97).

The sol-gel process consists of the formation of an inorganic network through the formation of a colloidal suspension (sol) and gelation of the sol to form a network in a continuous liquid phase (gel). In order to obtain systems containing SiO₂, such as glass, metal alkoxides are used as the precursors for synthesizing colloids. Alkoxysilanes, such as tetramethoxysilane (TMOS) and tetraethoxysilane (TEOS), are the most widely used alkoxides (98, 99).

Three reactions are generally used to describe the sol-gel process (100, 101): hydrolysis (Equation 1-6), water condensation (Equation 1-7) and alcohol condensation (Equation 1-8)



The silanols can then undergo polymerization reactions with other silanols or with other alkoxysilanes



In either case, for alkoxysilanes containing two or more alkoxy groups, the result is formation of a three-dimensional siloxane network throughout the liquid.

Several parameters influence the rate of the hydrolysis and polymerization reactions. These include temperature, pH, water concentration, and type of alkyl group used (101). An increase in temperature increases the rate of gelation. The hydrolysis reaction, through the addition of water, is very slow and entails the replacement of alkoxide groups with hydroxyl groups. It is most rapid and complete when catalysts

reaction, through the addition of water, is very slow and entails the replacement of alkoxide groups with hydroxyl groups. It is most rapid and complete when catalysts (acid or base) are used (102). The SiO_2 network formed under acid-catalyzed conditions are highly branched clusters. A larger, more sterically bulky alkyl group lowers the reaction rate (103).

Gels can be converted to silica through several routes. When the sol is cast into a mold, a wet gel will form. Thus, the colloid can be molded or dip coated into nearly any size or shape, including nanomaterials. The amount of acid/base present in the sol determines the gelling point of the system as well as various properties resulting from this gel (102). If the gel is heated or placed under vacuum to remove the solvent phase, typically alcohol, as well as water, the open three-dimensional network collapses, condensing it to a dense phase called a xerogel (97, 101). If the liquid in a wet gel is removed under supercritical conditions, a highly porous and extremely low density material called aerogel is obtained. The maximum temperature for either process can be kept under 100°C (103).

Sol-gel chemistry has been used to fabricate numerous template-synthesized nanostructures of TiO_2 and ZnO (27), V_2O_5 as a lithium ion battery material (104-107), SnO (108, 109) and WO_3 (110).

Silane coupling chemistry

Although bonding of organic polymers to inorganic surfaces has been a familiar operation (e.g., protective coatings on metals), a major need for new bonding techniques arose in 1940 when glass fibers were first used as reinforcement in organic resins (111). One relatively simple method for modifying surface chemistry is the use of

The general formula of an organosilane has two classes of functionality, $R_nSiX_{(4-n)}$, where X is a hydrolyzable group such as halogen, alkoxy, acyloxy, or amine. $n = 0$ for fully hydrolyzable groups. Following hydrolysis, a reactive silanol group is formed. The silanol group can condense with other silanol groups, for example, those on the surface of siliceous materials, to form siloxane linkages (111). Water for hydrolysis may come from several sources. It may be added, it may be present on the substrate surface, or it may come from the atmosphere (111). The R group is a nonhydrolyzable organic radical that may possess a functionality that imparts desired characteristics (111). The predominant type of organosilanes used for surface modifications are chlorosilanes and alkoxy silanes. Both are usually deposited from alcohol solutions.

Alkoxy silane chemistry is analogous to the chemistry of sol-gel production discussed above. The most common alkoxy groups are methoxy and ethoxy, which give methanol and ethanol as byproducts during coupling reactions. In the case of chlorosilane, the reaction with alcohol produces alkoxy silanes and hydrochloric acid (Equation 1-9).



The chlorine is the leaving group. Due to its high vapor pressure and high reactivity, tetrachlorosilane is used for vapor phase reactions to produce fumed silica (111).

Tetrachlorosilane has four reactive sites and thus can form three-dimensional network.

Silanes with one hydrolyzable group can be utilized to produce surfaces with monolayers. Because there is only one reactive site, these molecules can either dimerize or bind to the surface. Dimers cannot bind further and are rinsed away. Binding to the

or bind to the surface. Dimers cannot bind further and are rinsed away. Binding to the surface takes place via surface hydroxyl group (111). Silanes with one hydrolyzable group yield hydrophobic surfaces (111).

Silanes with two or more hydrolyzable groups (e.g., trichlorosilane or trialkoxysilane) are commonly used to produce surfaces with higher degrees of surface modifications. These silanes are first allowed to oligomerize in an aqueous alcohol solution (water content is typically around 5% vol/vol) in order to initiate the formation of silanols (111). The pH of the solution is adjusted to 4.5-5.5 with acetic acid to further facilitate the substitution reaction of the alkoxy-silanes (111). The materials such as glass (SiO_2) or metal oxides (Al, Zn, Sb, Ni) that need to be modified are then added to this solution and the oligomers bind through surface hydroxyl sites.

The degree of polymerization of the silanes is determined by the amount of water available and the organic substituent (111). The thickness of a polysiloxane layer is determined by the concentration of the siloxane solution. If a 2% solution of trialkoxy- or trichlorosilane is used, and five minutes is allotted for oligomer formation, the resulting surface modification is normally 3-8 monolayers thick (112). The silanized solution is then oven cured at 120 °C for 20-30 minutes or at room temperature for 24 hours, in order to remove the residual solvent.

Chlorosilane may also be employed to treat substrates under aprotic conditions. Toluene, tetrahydrofuran or hydrocarbon solutions containing 5% vol/vol silane are usually used (111). The materials that need to be modified are then added to this solution for 12-24 hours. The solvent is removed by drying in air and only a monolayer can form (111).

Electronically Conductive Polymers

Overview

Conventional insulating polymer systems have been increasingly used as substitutes for structural materials such as wood, ceramics, and metals because of their high strength, light weight, ease of chemical modification and customization, and processibility at low temperatures (113, 114). For the past 30 years, a special class of polymers has generated a great deal of interest due to their remarkable ability to conduct current. Reviews of these materials can be found elsewhere (115-117). In 1977, the high electrical conductivity of an organic polymer, polyacetylene, was reported (118, 119). Polyacetylene is typically synthesized by the polymerization of gaseous acetylene monomer using a soluble Ziegler-Natta catalyst (120). The observed increase in the electrical conductivity of polyacetylene when it was doped with iodine or other electron acceptors by nearly 10 orders of magnitude spurred interest in conducting polymers (114). Its conductivity was higher than for any known polymer and was comparable to that of copper. However, despite a high conductivity (10^3 S cm^{-1}), polyacetylene is instable in atmospheric conditions and this constitutes a major obstacle to practical applications.

The process of transforming a polymer to a conductive form by chemical oxidation or reduction is referred to as “doping”. Conducting polymers contain electronic states that can be reversibly occupied and emptied with electrochemical techniques. This ability to switch back and forth from a conducting to an insulating state by the application of a potential has enormous potential for wide-ranging practical applications of conducting polymers (e.g., batteries, capacitors, displays, chemical sensing applications (121), anticorrosion inhibitors (122, 123)).

The concept of conductivity and electroactivity of conductive polymers was quickly broadened from polyacetylene to include a number of other conductive polymers such as polypyrrole, polyalkylthiophene, polyaniline. Figure 1-8 shows the conductivities of some well-known materials, and are compared with those of conductive polymers.

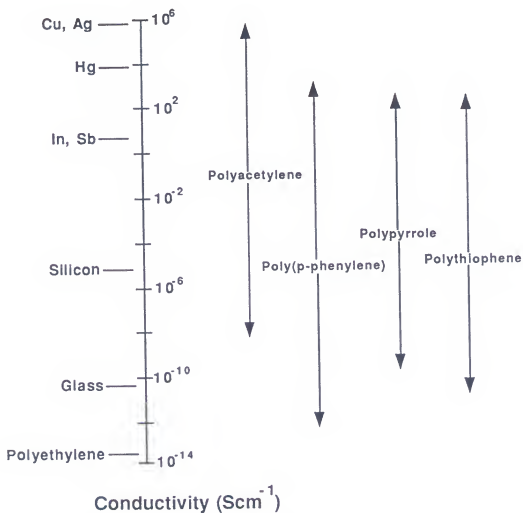


Figure 1-8. Range of conductivities of organic conducting polymers. Image adopted from B. B. Lakshmi, Ph.D. Dissertation, Colorado State University, Fort Collins, CO, USA, 1998.

Polyaniline (PANI)

Polyaniline is the oldest of the conducting polymers. References dating back to 1862 can be found describing a material known as "aniline black" (124). In the 1960s little research had been done on PANI, but in 1980s PANI became the center of considerable scientific interest and its electrically conducting properties became extensively investigated (125).

Owing to its low cost and excellent stability under a variety of conditions, PANI become an extremely well-studied conductive polymer (126, 127). PANI is generally prepared by direct oxidation of aniline using an appropriate chemical oxidant or by electrochemical oxidation at an electrode (128-133). Polyaniline can be present in five forms as leucoemeraldine, protoemeraldine, emeraldine, nigraniline and pernigraniline. Leucoemeraldine and pernigraniline are the fully reduced and the fully oxidized forms, and are not conducting. However, the intermediately oxidized emeraldine form exhibits the highest electrical conductivity.

The conductivity of PANI can vary from 10^{-13} to 350 Scm^{-1} , and it depends not only on oxidation level, but also on the pH of the solution in which PANI is equilibrated, as well as on the degree of the PANI fiber orientation. When it is synthesized with small anions it is insoluble in water and in most organic solvents. PANI is slightly soluble in pyridine and dimethylformamide and in concentrated sulfuric acid. The solubility of PANI can be significantly increased if synthesized using large, organic anions.

The potential applications of polyaniline include secondary batteries (134), molecular sensors (135), non-linear optical devices (136), electrochromic displays (137), microelectronic devices (138) and anticorrosion inhibitor (122, 123, 139, 140).

Chapter Summaries

Chapter 2 describes the transport of DNA molecules through nanopore membranes. We are interested in how pore diameter of the membrane affects rate and selectivity of DNA transport of different size and charge. Commercially available microporous polycarbonate membrane filters were used. DNA chains can be driven through the nanopore via the electrokinetic transport processes of electrophoresis and electroosmotic flow, as well as by diffusion. To our knowledge, there have been no quantitative studies of the relative importance of the electrokinetic and diffusive components for DNA transport in a nanopore system.

Chapter 3 describes a sol-gel template synthesis process that is used to produce silica nano-test-tubes within the pores of alumina templates. These silica nano-test-tubes are important because of the ease with which nearly any desired chemical or biochemical reagent can be covalently attached to their inside and outside surfaces. Inner and outer surfaces of the silica nano-test-tubes were functionalized using well-known silane chemistry. Green fluorescent silane was attached to the inner surfaces. The outer SiO_2 nano-test-tube surfaces were antibody functionalized using aldehyde methoxysilane linker. One of our key long-range objective is to develop nanotube technology for delivering biomolecules (e.g., DNA) to living cells.

Chapter 4 focuses on the mechanism by which polyaniline (PANI) films passivate stainless steel surfaces in highly corrosive H_2SO_4 solution. A variety of experimental methods including measurements of the open circuit potential, Auger depth profiling, and the scanning reference electrode technique (SRET) was used. These studies have shown that passivation is achieved because the oxidized and protically-doped emeraldine-salt form of PANI holds the potential of the underlying stainless steel electrode in the passive

region. Because of this electrostatic mechanism of corrosion inhibition, the entire stainless steel surface does not have to be coated with PANI in order to achieve passivation.

CHAPTER 2

DNA TRANSPORT IN A NANOPORE MEMBRANE

Introduction

The goal of this project is to monitor the transport of DNA molecules through nanopore membranes. DNA chains can be driven through the nanopore via the electrokinetic transport processes of electrophoresis and electroosmotic flow (EOF), as well as by diffusion. From fundamental point of view, we would like to understand how size, charge of DNA molecule and pore diameter of the membrane affect rate and selectivity of transport in nanoporous membranes. This work builds on pioneering studies of transport in such membranes done in the Martin group (34, 47-49, 58, 68, 69).

From a practical point of view, these studies should ultimately lead to membranes that can be used as molecular sieves to separate DNA molecules. Our long range objective is to develop membranes that can do practical chemical and bioseparation. Commercially available microporous polycarbonate membrane filters were used. Diffusion, electrophoretic and EOF fluxes are determined and compared. To our knowledge, there have been no quantitative studies of the relative importance of the electrokinetic and diffusive components for DNA transport in a nanopore system.

Background

There is tremendous current interest in transporting DNA molecules through nanopores (141-147). This interest stems from the possibility of using nanopores for characterization/sequencing (141) separation (142, 143) and sensing (144-147) of DNA. Nanopore systems being investigated include the α -hemolysin protein channel

(embedded in a lipid bilayer membrane) (141, 145, 146), nanopore filters (142), and microfabricated systems (144, 147). In the presence of an electric field, typically used in such applications, DNA chains can be driven through the nanopore via the electrokinetic transport processes of electrophoresis and electroosmotic flow, as well as by diffusion.

Deamer, Akeson and Branton et al. have found that single stranded DNA and RNA molecules can be driven electrophoretically through a 2-nm nanopore of an α -hemolysin channel in a lipid bilayer membrane (148-150). In fact, they went a step further and they detected these molecules as they were driven through a nanopore by an applied electric field (148-151). They demonstrated that this nanopore can discriminate between pyrimidine and purine segments along an RNA molecule.

Problem arises from the pore structure of these membranes. They are composed of a complex protein embedded in a lipid bilayer and are intrinsically unstable (148-152). The first question to be addressed is what kind of pore could be used. Polycarbonate membranes are more robust in this regard.

Experimental

Polycarbonate Membranes

A commercially available nanopore membrane with nominally 30-nm diameter pores was used (Osmonics, 9×10^8 pores per cm^2 , 6 μm thick). Membrane samples for the transport experiments were prepared by sandwiching 10 mm x 10 mm pieces of membrane between two 30 mm x 40 mm pieces of strapping tape (3M) (Figure 2-1). Each piece of tape had a 4.8 mm diameter hole punched through it, which defined the area of the membrane through which DNA transport occurred.

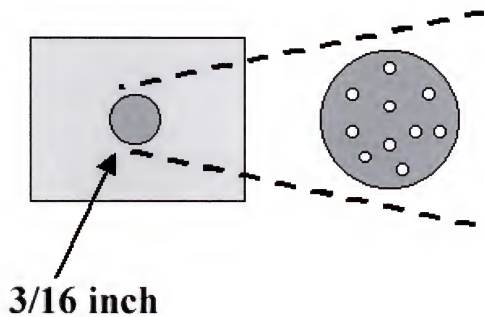


Figure 2-1. Scheme of polycarbonate membrane sandwiched between two scotch tapes.

Electrochemical Setup

The membrane sample was mounted between the two halves of an electrochemical U-tube permeation cell (Figure 2-2) (26). A Pt wire was inserted into each half-cell solution; the Pt wires were sheathed in tubes of the anionic polymer Nafion® (du Pont) to prevent contact between the DNA molecule and the electrode surface. Electrokinetic transport was driven by passing a 10 mA ionic current through the membrane. The cathode was in the feed half-cell and the anode in the permeate half-cell. The rate of transport of the DNA molecules was monitored by continuously circulating the permeate solution through a UV-visible detector (Hitachi Inc.) operating at 260 nm.

The DNAs were single-stranded homo-oligonucleotides made of thymidine bases (poly(T)_x), $x = 8, 24, 32$ and 64 (Integrated DNA Tech.). The feed half-cell contained 5 mL of a solution that was 2×10^{-5} M in the desired poly(T)_x dissolved in 0.2 M phosphate buffer, pH = 6.64. The permeate half-cell initially contained only this buffer solution.

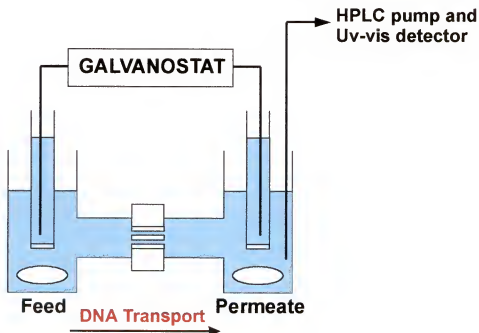


Figure 2-2. Electrochemical U-tube permeation cell.

Results and Discussion

It has recently been shown that the pores in these membranes do not have a constant diameter through out the membrane thickness (153). Instead, the pores are cylindrical through most of the membrane thickness, but taper to conical constrictions at both membrane faces; i.e. cigar-shaped pores (153). The geometry of the pores in the membranes used here was determined by electrolessly plating Au nanowires within the pores, dissolving the membrane in methylene chloride, and imaging the liberated nanowires using transmission electron microscopy (73). The radii of gyration for the poly(T)_x's were calculated using the Kratky-Porod equation (Equation 2-1) (154, 155) valid for wormlike polymer chains (154, 156).

$$r_g \approx \sqrt{\frac{pL}{3} \left[1 - 3\left(\frac{p}{L}\right) + 6\left(\frac{p}{L}\right)^2 - 6\left(\frac{p}{L}\right)^3 (1 - e^{-L/p}) \right]} \quad (2-1)$$

L in Equation 2-1 is the contour length of the DNA chain, taken as 0.43 nm times the number of bases (157). The parameter p is the persistence length, which characterizes the flexibility of a linear macromolecule (157-160). Because these molecules are charged, p is dependent on the ionic strength, I.

Following Manning (160) we have used the equation $p = 1.6 + 0.28/I$ (where p is in nm); this equation is valid for high ionic strength solutions like that used here (161). This equation yields $p = 3$ nm for our solutions, which is significantly shorter than the length of the poly(T)_x chain for all the poly(T)_x's except poly(T)₈. Hence, the radii in Table 2-1 are good approximations for the poly(T)_x's with x greater than 8, with the accuracy increasing with the value of x. In contrast, poly(T)₈ must be regarded as rigid rod rather than a wormlike polymer chain, with rod length equivalent to $L \sim 3.4$ nm.

Nernst-Planck Equation

The following form of the Nernst-Planck equation describes the flux of a charged molecule in the presence of a transmembrane electric field (26, 161).

$$J = -D \frac{\delta C}{\delta x} - \frac{zF}{RT} DC \left(\frac{\delta \phi}{\delta x} \right) \pm C v \quad (2-2)$$

D and z are the diffusion coefficient and charge of the molecule, $\delta C/\delta x$ is the concentration gradient across the membrane, and $\delta \phi/\delta x$ is the transmembrane electric field gradient. F is the Faraday constant, R is the gas constant and T is the Kelvin temperature. The first term in Equation 2-2 is the diffusive flux (J_{diff}), and the second is the electrophoretic flux (J_{ep}). With our electrode configuration, J_{diff} and J_{ep} for the poly(T)_x are in the same direction - from the feed to the permeate. For nanopores that have fixed surface charge, the third term in Equation 2 is the electroosmotic flux (J_{eof}), and v is the electroosmotic velocity (26, 162). Because the polycarbonate membranes used here have negative surface charge, with our electrode configuration, J_{eof} is in the opposite direction as J_{diff} and J_{ep} (162). Hence, the total poly(T)_x flux, J_{tot} , can be written as

$$J_{tot} = J_{diff} + J_{ep} - J_{eof} \quad (2-3)$$

Figure 2-3 shows plots of moles of poly(T)_x transported vs. time, with and without the applied transmembrane current. In all cases, the experiment was started with the current on, yielding a high flux state. At the indicated times (Figure 2-2) the current was turned off, yielding a lower flux state. The current was then turned back on (Figure 2-2) to give the high flux condition again. The slopes of the line segments when the current was on provided the total flux, J_{tot} (Equation 2-3), for the poly(T)_x. The slope of the line segment when the current was off provided the diffusive flux, J_{diff} , for the poly(T)_x.

The J_{diff} and J_{tot} data obtained from such analyses are shown in Table 2-1. J_{diff} decreases with increasing size (base number) of the poly(T)_x, indicating, as would be expected, that these membranes act as size-based ultrafiltration membranes for the poly(T)_x's. The flux with the current on, J_{tot} , is in all cases greater than the flux with the current off. This clearly illustrates the importance of the electrophoretic term, J_{ep} (Equation 2-3), to J_{tot} . The contribution of J_{ep} can be quantified by calculating the electrokinetic enhancement factor $E_{\text{ek}} = J_{\text{tot}}/J_{\text{diff}}$ (Table 2-1) (26). The E_{ek} values show that electrophoresis dramatically enhances DNA transport, especially for the larger DNA chains.

Table 2-1. Radii of gyration (r_g) and transport parameters.

Base number, x	8	24	32	64
r_g (nm)	--	2.0	2.5	3.8
J_{diff} (nmole h ⁻¹ cm ⁻²)	5.2 ± 0.5	1.6 ± 0.2	1.5 ± 0.1	0.5 ± 0.1
J_{tot} (nmole h ⁻¹ cm ⁻²)	28 ± 2	36 ± 4	35 ± 2	22 ± 2
E_{ek}	5	23	23	44

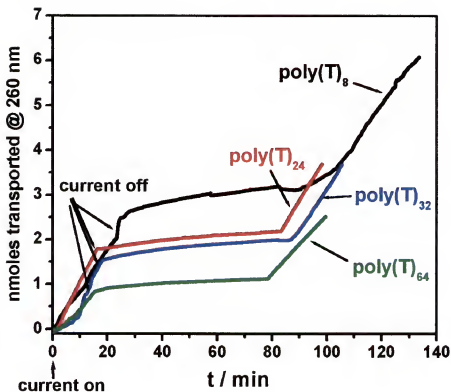


Figure 2-3. Plots of nanomoles transported vs. time with and without the applied transmembrane ionic current. Black = poly(T)₈. Red = poly(T)₂₄. Blue = poly(T)₃₂. Green = poly(T)₆₄.

The electrokinetic enhancement factor increases with size (base number) of the poly(T)_x chain because, to a first approximation, each additional base adds an additional increment of charge (z in Equation 2-2) to the chain. This means that the electrophoretic term becomes more important with increasing base number. There are however, two caveats here. First, note that the diffusion coefficient, D , also appears in the electrophoretic term, and as the J_{diff} data (Table 2-1) clearly show, D decreases with increasing base number. Hence, as bases are added we see two antagonistic effects – D decreases but z increases. The second caveat concerns the overly simplistic supposition

that each additional base increases z by 1. This is overly simplified because in electrolyte solution, the charge on the DNA chain is partially screened by the counterions of the electrolyte. Hence an 8-mer DNA chain, for example, will have an effective charge in solution that is significantly less than -8 . This interplay between the D and z terms is clearly seen in the J_{tot} data in Table 2-1. Note that J_{tot} initially increases with base number (z effect dominating) but ultimately decreases again for the longest chain (influence of decreased D).

The large values of E_{ek} also show that J_{ep} (from feed to permeate) is much greater than J_{eof} (in the reverse direction). If this were not the case, the flux with the current on would be lower than J_{diff} (Equation 2-3), and E_{ek} would be less than unity. Because any contribution of J_{eof} is overwhelmed by the contribution of J_{ep} , it is impossible to obtain an experimental measure of J_{eof} from these data.

We can however, make the following general comments. First, it is well known that increasing the ionic strength of the buffer used minimizes the contribution of EOF to the transport process (26). Indeed, we have shown that EOF in carbon nanotube membranes is almost completely eliminated when the buffer ionic strength is as high as that used here (26).

Second, the EOF rate is dependent on the charge density on the pore walls. With these membranes the anionic functionalities on the pore walls are not the result of intentional chemical functionalization; i.e., these are not cation exchange membranes. Rather, the anionic charge results from adsorption of anions to the pore walls and from the hydrolysis of the polycarbonate during pore formation (162). As a result, the charge density is low, and this also contributes to the lack of a significant EOF term here.

Finally, the high ionic charge of the DNA (one charge for each base) insures that the contribution of electrophoretic transport is high. For all of these reasons, it is not surprising that the dominant electrokinetic transport phenomena for DNA in these membranes is electrophoresis.

Conical Pores and Mass Transfer

The final issue addressed here concerns the effect of the conical pore ends on the rate of mass transfer in these membranes. Transmission electron micrographs of nanowires plated within the pores of the membrane clearly show the tapered conical pore ends (upper right in Figure 2-4). Analysis of many such images showed that the cylindrical part of the pore that runs through most of the membrane thickness is 55 ± 4 nm in diameter, and the tapered pore ends are 80 ± 20 nm long. Field emission scanning electron microscopic images of the surface of the membrane provided the diameter of the constriction at the membrane surface, 27 ± 8 nm. The key question is – do the short cone-like ends offer significant resistance to mass transfer relative to the much longer cylindrical pore that runs through most of the membrane thickness?

Because it is mathematically more tractable, we explore this issue by using the ionic resistance of the pore as an approximation for the steady-state mass-transfer resistance. The ionic resistance of an electrolyte-filled cone (R_c) is given by (163).

$$R_c = \frac{4 \cdot \rho \cdot l}{\pi \cdot d_b \cdot d_t} \quad (2-4)$$

where ρ is the resistivity of the electrolyte, l is the length of the cone (80 nm), d_b is the diameter at the base of the cone (55 nm), and d_t is the diameter at the tip of the cone (27 nm).



Figure 2-4. TEM image an Au nanowire plated within a pore of the membrane showing the conically-shaped end.

Plugging these values into Equation 2-4 gives $R_c = (7.3 \times 10^5)\rho$. However, since there are two conical tapers (one at each membrane face), the total cone resistance for this membrane is $R_{\text{ctot}} = (1.4 \times 10^6)\rho$. The ionic resistance of an electrolyte-filled cylindrical pore (R_{cyl}) is given by,

$$R_{\text{cyl}} = \frac{\rho \cdot l}{\pi \cdot r_{\text{cyl}}^2} \quad (2-5)$$

where r_{cyl} is the radius of the cylindrical pore. These membranes are 6 μm thick, but there is an 80 nm-long conical taper at each membrane face; hence, the length of the cylindrical part of the pore in these membranes is $l = 5.84 \mu\text{m}$. With this value of l and $r_{\text{cyl}} = 27.5 \text{ nm}$, Equation 5 gives $R_{\text{cyl}} = (2.4 \times 10^7)\rho$ for the cylindrical part of the pore. Ratioing R_{ctot} and R_{cyl} shows that the resistance to mass transfer in the cone-like pore ends is $\sim 6\%$ of the mass-transfer resistance of the longer cylindrical part of the pore. Hence, the net membrane mass-transfer resistance is dominated by transport in the cylindrical part of the pore, and to a first approximation, the mass-transfer resistance of the conical pore ends is negligible.

There is, however, a caveat. If the diameter of a molecule was comparable to the diameter of the constriction at the membrane face (27 nm), then the molecule could experience hindered diffusion at the constriction (164). This would accentuate the resistance to mass-transfer at the constriction. In the limit, one could envision, for example, a 35 nm diameter molecule that could pass through the central part of the pore (diameter = 55 nm) but is prevented from doing so by the 27 nm-diameter constriction at the membrane face. In this case, the mass-transfer resistance of the cone would be infinite.

However, DNA and other linear macromolecules offer an interesting solution to this problem – they can unfold from their random coil configuration and reptate through a nanopore. Hence, for such molecules it seems that it will typically be the case that the mass-transfer resistance of the cylindrical part of the pore will dominate. This issue of a constriction in a nanopore is not unique to these membranes. For example, there is an analogous constriction in the lumen of the α -hemolysin protein channel (141). It would be interesting to use an approach based on Equations 2-4 and 2-5 and the dimensions of the lumen and the constriction to see if the constriction presents the dominant mass transfer resistance in this channel.

Because the membrane used here has a high pore density, the transport data are an average over many pores. However, single-pore versions of these membranes can be prepared, as can membranes that have conical pores that run through the entire membrane thickness (163). Furthermore, we have shown that the inside diameter of such pores can be controlled at will, down to molecular dimensions, by plating gold nanotubes within the pores (165). As a result, the inside diameter can be made comparable to the lumen of a protein channel. In addition, the Au nanotubes can be chemically modified via thiol chemisorption, which allows the chemistry and charge of the pore wall to be varied at will (165). Using the analyses developed here with such single conical nanotube-containing membranes should provide good model systems for studying electrokinetic biomolecule transport in protein channels.

Conclusions

We have shown that single-stranded homo-oligonucleotides can be driven through the nanopore via the electrokinetic transport processes of electrophoresis and diffusion.

J_{diff} decreases with increasing DNA base number indicating that these membranes act as size-based ultrafiltration membranes. The total flux with the current on is in all cases greater than the flux with the current off. The high ionic charge of the DNA insures that the contribution of electrophoretic transport is high. The EOF rate is dependent on the charge density on the pore walls. In our case the charge density of the pore walls is low which contributes to the lack of a significant EOF term. Thus, the dominant electrokinetic transport phenomena for DNA in these membranes is electrophoresis. The E_{ek} values show that electrophoresis dramatically enhances DNA transport, especially for the larger DNA chains. Transmission electron microscopy results showed that pores in polycarbonate membranes are cigar-shaped. Thus, they do not have a constant diameter through out the membrane thickness. This is probably due to inhomogeneous morphological structure of the finite crystalline domains and an asymmetric manufacturer's processing of the film surfaces (153).

CHAPTER 3 PREPARATION AND MODIFICATION OF SILICA NANO TEST TUBES

Introduction

We have been investigating potential biomedical and biotechnological applications of template-synthesized nanotubes. One application we envision for these nanotubes is as vehicles for delivery of drugs, DNA, proteins or other biomolecules. For such applications, it would be best to have nanotubes that are closed on one end and open on the other – nano test tubes. We describe here a simple modification of the template-synthesis method that allows for the preparation of nano test tubes. Prototype silica nano test tubes are discussed here. We show that the outside diameter of these test tubes is determined by the pore diameter in the template membrane used and that the length of the test tube is determined by the thickness of the template membrane. The ability with template synthetic method to control the dimensions of the nano test tubes creates the opportunity to tailor nanotubes specifically to the biomedical problem at hand. We also show that inner and outer silica nano test tube surfaces can be easily chemically and biochemically functionalized.

Background

Nanoparticles in Biomedical Research and Development

There is enormous current interest in using nanoparticles for biomedical applications including enzyme encapsulation (166), DNA transfection (167-170) and drug delivery (171). Typically, spherical nanoparticles are used for such applications because spherical particles are easy to make. Self-assembling lipid tubules have also

been used in biomedical applications although it is difficult to control tubule diameter or length and the lipid tubules must be coated with ceramic or metal to make them rugged enough for biomedical use (172).

The Martin group has developed technologies for preparing highly monodisperse tubular nanoparticles of any size and composed of nearly any material, or combination of materials (24-27, 53). These tubular nanostructures have unique attributes, relative to nanospheres, that make them ideal candidates for many biomedical applications. These tubular structures have large inner volumes (relative to the dimensions of the tube), which can be filled with any biological/biochemical entity from cells, to proteins, to small molecules. Such nanotubes have distinct inner and outer surfaces and their open geometry (i.e., they have mouths) make accessing and functionalizing these surfaces particular easy.

Furthermore, with the template synthesized nanotubes it is easy to apply different chemical and/or biochemical functional groups to the inside vs. outside surfaces. Martin et al. have shown that the nanotubes modified with C_{18} on the inside and unmodified on the outside are affective at sorbing hydrophobic drugs and drug analogs from aqueous solution (53). Multiple different chemical/biochemical species can be applied to a given nanotube surface. This raises the possibility of creating multiple chemical and biochemical sub-phases within a single nanotube. Thus, the ability with Martin's template synthetic method to control the dimensions and chemistries of the nanotubes creates the opportunity to tailor nanotubes specifically to the biomedical problem at hand. Finally, the ability to make these nanotubes out of nearly any material or combination of

materials opens the possibility to create tubes with desired biochemical properties such as biocompatibility or biodegradability (24).

Drug delivery and DNA transfection

A recurrent theme in the recent literature involving nanoparticles concerns their use as drug delivery agents (171, 173-175). One approach for making nanoparticles for drug delivery entails the use of block copolymers (176-178). Linking together small monomer molecules forms polymers. There are two major synthetic routes to prepare block copolymers. The first one is the copolymer micelle formation by dissolving the block copolymer directly in water or other aqueous solvent (e.g., buffer), and dialysis (177, 179, 180). The second synthetic route is the preparation of dendrimers using dendrimer chemistry (181). Compared to polymer micelles, dendrimers are very highly monodisperse and have precisely defined chemistries.

There are several advantages of using nanoparticles for drug delivery. Paul et al. found that using nanoparticles to deliver drugs increased the drug efficacy versus the free drug (182). Perkins et al. found that the nanoparticles can reduce drug toxicity (175). Encapsulation in nanoparticles can also protect fragile drugs from severe gastrointestinal conditions and allow alternate means of treatment (183).

The second recurrent theme in the recent literature involving nanoparticles concerns their use for DNA transfection (166-170). DNA transfection is the process by which DNA is delivered from the exterior to the interior of a cell and ultimately to the cell nucleus for protein expression (184). Many different vehicles are being investigated. These include calcium phosphate precipitates of plasmid DNA (185, 186), cationic liposomes of plasmid DNA and oligonucleotides (187-189), and viruses (190), such as

adenovirus (191) and retroviruses (192). Gene therapy in whole animal studies has centered on the use of liposomes (193, 194) and viruses (190-192).

Each of these delivery methods has drawbacks that make them less than ideal. The cost, especially for viruses, can be quite high. In addition, there is very little cell selectivity with liposomes and viruses, unless there is a specific interaction. Furthermore, viral-coat proteins can be immunogenic, as in the case with adenovirus, which causes significant cellular and immunologic reactions, the most severe being death (195, 196).

Nanotubes prepared in Martin group offer interesting opportunities for gene delivery. For example, with viral transfection agents each virus contains only one copy (for DNA viruses) or two copies (for RNA viruses) of the genetic material. In contrast nanotubes can in principle be packed with many copies of supercoiled plasmids. Furthermore, highly monodisperse nanotubes of any size and composed of nearly any material can be prepared by the template method, and nanotubes that are the sizes of virus particles (~ 100 nm) are easily made. However, in order to use nanotubes as DNA transfection vehicles that deliver genetic material to specific desired cell types, technologies for preparing nanotubes with nanoparticle caps must be developed. Such nanotubes need to lose their caps, thus opening the nanotube and making the “payload” available, when the assembly is partitioned into a cell.

Here we demonstrate that highly monodisperse nano test tubes can be prepared. The advantages of these nano-test-tubes are in one side of the test-tube being closed and the other side being open. They are hollow (*vide infra*) and thus can be packed with biomolecules (e.g., DNA). We show that the outside diameter of these test tubes is determined by the pore diameter in the template membrane used and that the length of the

test tube is determined by the thickness of the template membrane. We also show that inner and outer SiO₂ nano test tube surfaces can be chemically and biochemically functionalized.

Experimental

Tetraethoxysilane and 3-trimethoxysilanealdehyde (United Chemical Technologies, Inc.), N-(triethoxysilylpropyl)-dansylamide (Gelest, Inc.), ethanol (Fisher), HCl (Fisher), CH₃COOH (Fisher), NaOH (Fisher), phosphoric acid (Fisher), KCl (Fisher), NaCl (Fisher), toluene (Fisher), dichloromethylene (Fisher) were used as received.

Purified water (obtained by passing house-distilled water through a Millipore water purification system) was used to prepare buffer solutions. Commercial Anopore alumina membranes used for vacuum filtration were obtained from Whatman (Clifton, NJ). These were disks 13 mm in diameter with cylindrical pores of nominally 20 nm diameter. Alumina filter membranes of 70 nm pore diameter were grown in house by anodic oxidation of high purity aluminum (Figure 3-1). Unless otherwise noted, templates with 600, 800 nm and 1.2 μ m thickness were used for our studies.

1 mg/mL of Anti-ICAM-1 human (mouse) antibody (Calbiochem) was dissolved in PBS buffer (50 mM sodium phosphate, 100 mM KCl, 150 mM NaCl, pH 7.5), pipetted into 1 mL vials to obtain 100 μ g/mL and stored at -80 °C to avoid freeze / thaw cycles.

Scanning electron microscopy was performed using a Hitachi S-4000 field emission scanning electron microscope (FESEM). FESEM samples were made conductive by sputtering a thin layer of gold-palladium alloy using a Denton Vacuum Desk II model sputter coater. Transmission electron microscopy (TEM) was performed on a Hitachi H-7000 TEM instrument. Fluorescence microscopy was performed on a

Bio-Rad MRC 1024 laser scanning confocal microscope. Excitation frequency of 522 nm was used.

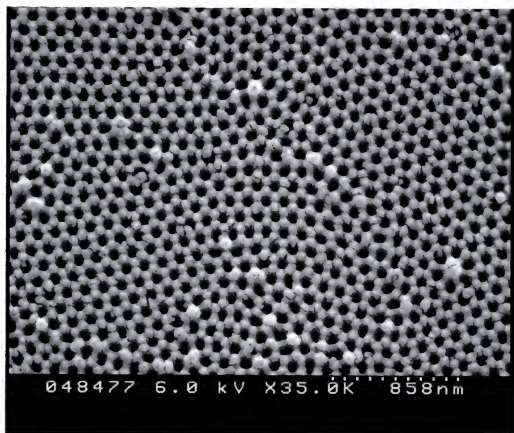


Figure 3-1. Scanning electron micrograph of homegrown alumina template membrane (70 nm dia, 600 nm thickness). Courtesy image of Ms. Lacramioara Trofin.

Template Synthesis

A sol-gel template process was used to prepare nano-test-tubes (110). See Chapter 1 for a review of the chemistry involved. The sol precursor solution was prepared by mixing 50 mL of absolute ethanol, 5 mL of tetraethylorthosilicate (Figure 3-2A) and 1 mL of 1 M aqueous HCl. This solution was allowed to hydrolyze for 30 minutes. The alumina template membrane was then immersed into this sol with sonication for 1 min, and allowed to remain in the sol for an additional 3 min without sonication. The sol-impregnated membrane was then dried in air for 10 minutes and oven cured overnight at 150 °C.

This method yields the desired silica nano test tubes within the pores of the membrane plus a thin surface silica film covering the face of the membrane. This surface film was removed by wiping the membrane surface with a laboratory tissue soaked in ethanol. This removed the surface layers of SiO₂ that would otherwise bind the nanotubes together. It is important to note that in order to prepare silica test tubes the template alumina membrane is not free standing *i.e.* the barrier layer of the alumina membrane is still attached to the underlying aluminum. See Chapter 1 for the review of alumina growth and barrier layer. The template membrane was then dissolved by overnight immersion into a 25% (wt/wt) solution of H₃PO₄. The liberated test tubes were collected by filtration and rinsed with copious quantities of pH 7.0 phosphate buffer and water.

Silanization and Antibody Modification

Structures of all silanes used for surface modification are shown in Figure 3-2. The inner walls of the nano-test-tubes were functionalized by immersing the SiO₂-coated alumina membrane in a 15% N-(triethoxysilylpropyl)-dansylamide silane (Figure 3-2B)

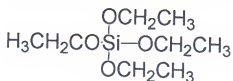
solution (vol/vol) in ethanol. The silica nano-test-tubes were still embedded within the pores of the alumina template membrane. The deposition solution was stirred for 30 minutes before addition of the membrane to allow formation of siloxane oligomers. Following deposition, the template membrane was thoroughly rinsed with ethanol, briefly dried in air and oven cured for 20 min at 150 °C.

Attaching the first silane while the test-tubes are still embedded within the pores masks the outer tube surface. In order to expose the outer tube surface, the silica nano-test-tubes with attached green fluorescent silane (Figure 3-2B) were liberated by dissolving the alumina template membrane overnight in a 25% (wt/wt) solution of H_3PO_4 . The nano test tubes were filtered from the acid solution using commercially available alumina filters (pore diameter 20 nm) and repeatedly rinsed with pH 7.0 phosphate buffer and water, then dried. The nano test tubes were then dispersed in ethanol by sonicating the alumina filter membrane for 2 hours.

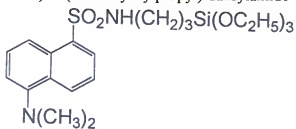
Antibody functionalized SiO_2 nanotubes are typically prepared using an aldehyde terminated siloxane linker (197). The nano test tubes with the green fluorescent silane attached to the inner surfaces were stirred in a 5% aqueous, 10% aldehyde methoxysilane (Figure 3-2C) in ethanol, pH adjusted to 5.0 with acetate for 20 minutes. The aldehyde-modified nano test tubes were filtered, rinsed with ethanol, and dried 24 hours in an oxygen free glove box. After drying, the nano test tubes were incubated with a solution of Anti-ICAM-1 human (mouse) antibody in PBS buffer (1mg antibody/ml buffer). Incubation was carried out at 4° C overnight. Aldehydes have been shown to react with pendant primary amines on antibodies to covalently link the antibodies to the

substrate (198-200). This resulted in antibodies being bound to the outer surface of the nano test tubes.

A) Tetraethylorthosilicate (TEOS)



B) N-(triethoxysilylpropyl)-dansylamide



C) 3-trimethoxysilanepropaldehyde

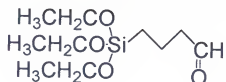


Figure 3-2. Structures of silanes used for modifications.

Filling the Nano-Test-Tubes

We wanted to prove that the silica nano test tubes obtained were, indeed, hollow. We have shown previously that if a polymer solution is applied to the surface of a nanopore template membrane, the solution coats the pore walls, and after evaporation of the solvent, polymeric nanotubes are obtained (24). We reasoned that if the silica nano test tubes are hollow, they could be used as templates in this way to prepare polymeric nanotubes. A 4% (wt/wt) polystyrene solution was prepared in 50:50 (vol/vol) toluene/dichloromethane. A drop of this solution was placed onto the surface of a silica nano test tube-containing template membrane and the solvent was allowed to evaporate. The template membrane was then dissolved in 1 M NaOH yielding a film of polystyrene. The surface of this film that was in contact with the membrane was then imaged with the FESEM to see if polystyrene nanotubes (formed in the hollow silica nano test tubes) protrude from this surface. If polystyrene nanotubes are observed, then the nano test tubes must be hollow.

Results and Discussion

Differential Inside / Outside Modification

Perhaps the most important attribute of the nanotubes is their distinct inner and outer surfaces, which can be independently chemically and biochemically functionalized. The template method provides a very simple way to differentially modify the inner vs. outer surfaces. This concept is illustrated in Figure 3-3.

While still embedded within the pores of the template membrane, the inner surfaces are reacted with the first silane. The outer surfaces are masked because they are in contact with the pore walls of the template membrane. The amphoteric alumina template

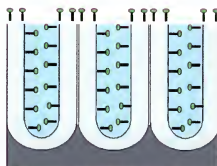
1. Cross-section of Al_2O_3 template membrane



2. Sol-gel synthesis of SiO_2 test-tubes



3. Attach first silane to inner test-tube surfaces

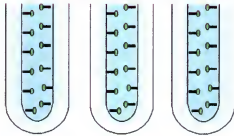


Green fluorescent silane is attached to silica that was deposited on to alumina surface and pore walls.

Figure 3-3. The concept of chemical and biochemical test-tube functionalization.

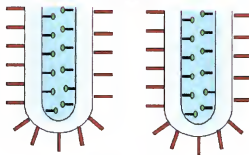
↓
4. Remove surface films and dissolve template membrane

LIBERATED NANO-TEST-TUBES



Silica test tubes with attached green fluorescent silane are shown.

5. Attach second silane to outer surfaces



↓
6. Antibody attachment to outer surfaces

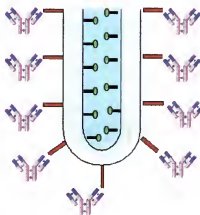


Figure 3-3. Continued

is then dissolved with acid or base to liberate the silica test tubes. These are collected by filtration, resuspended and reacted with the second silane.

This second silane attaches to the now accessible outer surfaces of the silica test tubes. As conclusive proof of independent inside/outside modification, we chemically modified the interior and biochemically modified the exterior of the silica nano test tubes. We covalently attached fluorescent green silane N-(triethoxysilylpropyl)-dansylamide (Figure 3-2B) to the inner surfaces of the silica nano test tubes. The outer SiO₂ nano test tube surfaces were antibody functionalized using propaldehyde methoxysilane linker (Figure 3-2C).

ICAM is a cell-specific antibody. Cell-surface specific antigens exist on many cell types. Endothelial cells express intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) which are responsible for attachment and transmigration of white blood cells across the tight junctions into the underlying tissues (201-205). The antibody attached to the outer surfaces of the silica nano test tubes was anti-ICAM-1 human (mouse) monoclonal antibody, the endothelial cell-specific antigen.

This presents a very elegant model to test the cell selectivity of the transfection process mediated by the antibody-coated-nano test tubes. Our selective antibody-coated nano test tubes have a potential to make them highly targetable to specific cells, a feature typically lacking with viruses used for gene therapy.

Fluorescently modified nano-test tubes

The outer diameter of the silica nano test tubes can be controlled by varying the pore diameter of the alumina template membrane, the length can be controlled by varying the thickness of the template. Figure 3-4 shows TEM image of silica nano test tube that is 70 nm in diameter and ~ 600 nm in length filled with fluorescent green silane. The

mouth and the bottom of the silica nano test tube are clearly seen. Figure 3-5 shows the bottom (round part) of the silica nano test tube.

We show also here that different sizes of nano test tubes can be prepared (Figure 3-6). To prove the concept that our nano test tubes are indeed hollow, we used polystyrene solution to fill the pores of the nano test tubes that were still embedded within the pores of the alumina template membrane. Thus, we created the reversed matrix of the silica nano-test-tubes. Figure 3-7 shows SEM image of the polystyrene that was used to fill the nano test tubes.

To prove the concept that we can prepare fluorescently labeled nano-test-tubes, we covalently attached fluorescent green silane N-(triethoxysilylpropyl)-dansylamide to the inner surfaces of the silica nano test tubes. Nano test tubes with attached green fluorescent silane were filtered from the acid solution using commercially available alumina filters and repeatedly rinsed with pH 7.0 phosphate buffer and water, then dried. The nano test tubes were then dispersed in ethanol by sonicating the alumina filter membrane for 2 hours.

100 μ L of this solution was applied to a glass cover slip (Fisher, Premium cover slip 18 x 18 mm) and let dry in air for 2 days. The slide was covered all the time with aluminum foil to avoid direct indoor light with fluorescently labeled nano test tubes.

The glass slide was previously thoroughly cleaned by sonicating it first in absolute acetone and then in house-distilled Millipore water for 5 minutes. This step was repeated three times. Finally, the slide was dried under N₂. Confocal fluorescent microscope was used to detect the green fluorescent nano test tubes. A fluorescence image of



Figure 3-4. TEM image of silica nano-test-tube prepared within homegrown alumina membrane.

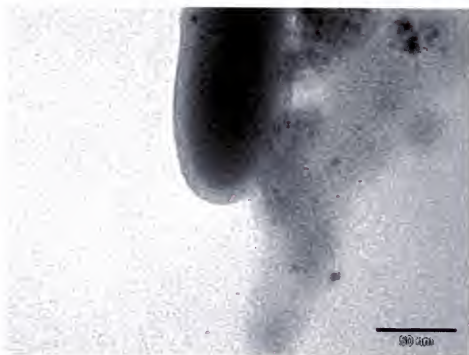


Figure 3-5. TEM image of round part of silica nano test tube.

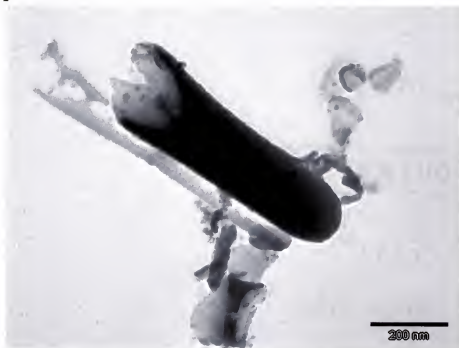
A**B**

Figure 3-6. TEM images of different sizes of silica nano test tubes.

C



Figure 3-6. Continued

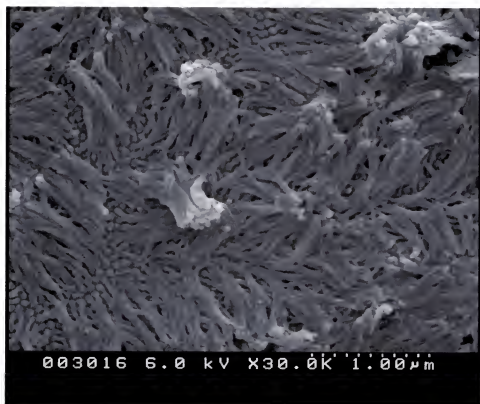


Figure 3-7. Scanning electron micrograph image of polystyrene tubes.

A

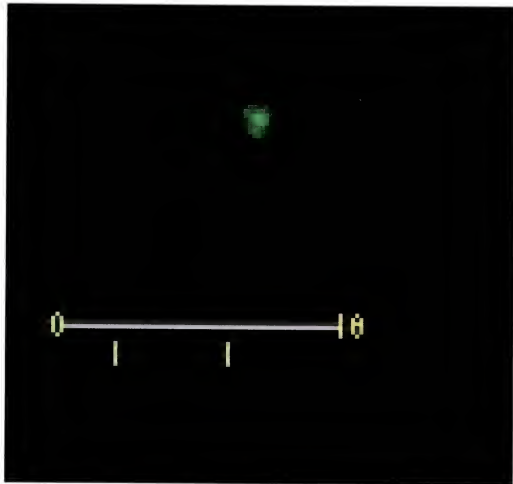


Figure 3-8. Confocal microscope image of 70 nm dia silica nano test tube. Tube thickness = 600 nm. The scale bar = 10 μm .

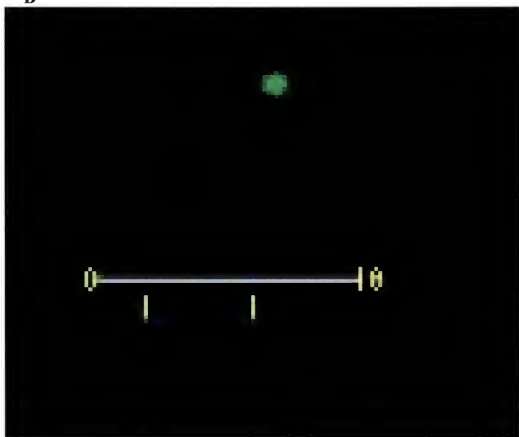
B

Figure 3-8. Continued.

fluorescently modified nano test tubes (70 nm dia, ~ 600 nm length) are shown in Figures 3-8A and B. Due to their small size, nano test tubes can be seen only as dots.

Antibody modified nano-test-tubes

Anti-ICAM-1 human (mouse) monoclonal antibody was used to biochemically modify the exterior of the silica nano-test-tubes. Biotinylated Anti-ICAM-1 was used to attach streptavidin-gold nanoparticles (10 nm) onto the outer surfaces of silica nano test tubes. This concept is shown in Figure 3-9. Figure 3-10A shows TEM image of unmodified (Figure 3-10A) and biochemically modified (Figure 3-10B) silica nano test tubes. It is clearly seen that gold nanoparticles are present on the nano test tube surface (Figure 3-10B). Thus, our biochemically modified nano test tubes can have potential applications in targeted drug delivery by binding of the antibody to a specific antigen on a particular population of cells. However, the success of targeted drug delivery depends on sufficient attachment of antibody to the silica surface. We should explore other antibody attachment strategies to silica surface in order to find desirable attachment of antibody.

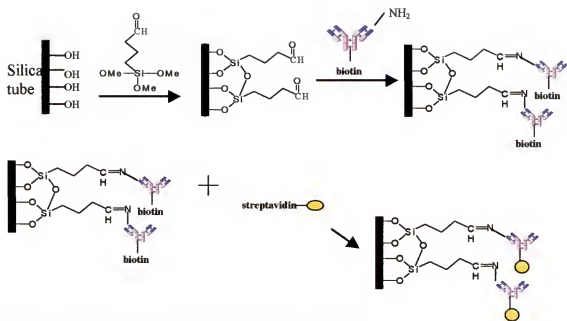


Figure 3-9. The concept of antibody attachment strategy.

A**B**

Figure 3-10. TEM image of silica nano test tube without (A) and with attached antibody (B).

Conclusions

A sol-gel template synthesis process was used to prepare silica nano test tubes within the pores of alumina template membranes. We have shown that these test tubes are closed on one end, open on the other, and hollow. The length of these test tubes can be controlled at will by varying the thickness of the template membrane used. We have also shown that that silica nanotubes can be chemically and biochemically functionalized using silane chemistry with commercially available reagents. The inner surfaces of the silica nano test tubes were functionalized with green fluorescent silane and the outer surfaces of the silica nano test tubes were tagged with antibodies that promote uptake by endothelial cells. The final challenge to be addressed if these test tubes are to be used for biomolecule delivery is to develop procedures for capping and uncapping the open mouth.

CHAPTER 4 CHARACTERIZATION OF POLYANILINE (PANI) PROPERTIES

Introduction

The objective of this work was to elucidate the mechanism by which polyaniline (PANI) films passivate stainless steel surfaces in highly corrosive H_2SO_4 solution. A variety of experimental methods including measurements of the open circuit potential, Auger depth profiling, and the scanning reference electrode technique (SRET) was used. These studies have shown that passivation is achieved because the oxidized and protically-doped emeraldine-salt form of PANI holds the potential of the underlying stainless steel electrode in the passive region. Because of this electrostatic mechanism of corrosion inhibition, the entire stainless steel surface does not have to be coated with PANI in order to achieve passivation. Auger depth profiling experiments show that, in analogy to the case of a bare stainless steel surface whose potential is potentiostatically maintained in the passive region, the stainless-steel surface beneath the PANI-film is enriched in Cr after exposure to the H_2SO_4 solution. Finally, SRET was used to explore passivation of the stainless steel in regions exposed to solution by pinholes intentionally cut through the PANI film. SRET shows that the PANI film surrounding the pinhole can heal the stainless steel in the pinhole region.

Background

Polyaniline (PANI) is one of the most intensively investigated of the electronically conductive polymers (205-211). In 1985, DeBerry found that PANI films electrodeposited on stainless steel passivated the surface to corrosion in sulfuric acid

solution (212). Since then there have been numerous studies of the corrosion inhibition properties of PANI (140, 213-217) and other electronically-conductive polymers (218). Nevertheless, there is no universally agreed upon mechanism by which these polymers provide anticorrosion properties. We have investigated corrosion inhibition by chemically-synthesized PANI films coated onto stainless-steel surfaces in aqueous sulfuric acid solution. The emeraldine-base form of the polymer was coated onto the electrode surface; however, exposure to the H_2SO_4 solution converted this to the protically-doped emeraldine salt form.

A variety of experimental methods including measurements of the open circuit potential, Auger depth profiling, and the scanning reference electrode technique (SRET) was used to explore the mechanism by which the PANI film passivates the underlying stainless-steel surface. These studies have shown that passivation is achieved because the PANI film holds the potential of the underlying stainless steel electrode in the passive region. The Auger results lend especially strong support to this conclusion. In analogy to the case of a bare stainless steel surface whose potential is potentiostatically maintained in the passive region, the stainless-steel surface beneath the PANI-film is enriched in Cr after exposure to the H_2SO_4 solution. Because the PANI film passivates the electrode by simply holding the potential in the passive region, the entire stainless steel surface does not have to be coated with PANI in order to achieve passivation; studies on partially-coated electrode surfaces support this model. SRET was used to explore passivation of the stainless steel in regions exposed to solution by pinholes intentionally cut through the PANI film. SRET shows that the PANI film surrounding

the pinhole can heal the stainless steel in the pinhole region. The results of these investigations are reported here.

Experimental

Materials

Aniline (Aldrich) was vacuum distilled prior to use. N-methylpyrrolidone (Sigma), HCl (Fisher), NH_4OH (Fisher), ethanol (Fisher) and $(\text{NH}_4)_2\text{S}_2\text{O}_8$ (Aldrich) were used as received. Purified water (obtained by passing house-distilled water through a Millipore water purification system) was used to prepare all solutions. The composition of the ferritic stainless steel SS-430 samples (as specified by the provider, Metal Samples Company) is shown in Table 5-1.

Table 4-1. Composition of the SS-430 stainless steel.

C	Ni	Cr	P	S	Mn	Si
0.048%	0.150%	17.320%	0.032%	0.009%	0.490%	0.356%

Polymer Synthesis

PANI was chemically synthesized according to the procedure of MacDiarmid et al (211). Briefly, a solution that was 1 M in aniline and 1M in HCl was mixed with an equal volume of a solution that was 1 M in $(\text{NH}_4)_2\text{S}_2\text{O}_8$ and 1M in HCl. This results in oxidative polymerization of the aniline to produce the protically-doped, emeraldine salt form of PANI (211). The polymerization was allowed to proceed overnight. The resulting green precipitate was collected by filtration, rinsed with water and dried in vacuo. The emeraldine salt was deprotonated by dispersing the insoluble powder in excess 1M aqueous NH_4OH . This converted the emeraldine salt into the blue emeraldine base, which is also insoluble in water. The emeraldine base was then collected by filtration, rinsed with water and methanol and dried in vacuo. One g of the emeraldine

base was then added to 100 ml of N-methylpyrrolidone (NMP). The resulting solution was stirred overnight and then filtered to remove the small amount of insoluble material. The final concentration of PANI was 3.4 % (w/w).

Electrode Preparation

The electrodes were 1.6 cm-diameter stainless steel disks which were finished prior to use with 600 grit SiC paper on a polishing wheel. The electrodes were then rinsed with Millipore water and ethanol and dried in air. The electrodes were coated with PANI by applying measured volumes of the emeraldine base solution (in NMP) to the electrode surface using a microliter syringe. The solvent was allowed to evaporate in air at room temperature; the time required for evaporation depended on the volume applied and ranged from one day to four days. The thickness of the PANI film was determined using a Dektak 3030 profilometer. This was accomplished by removing half of the PANI film from the stainless steel surface using a razor blade and scanning the profilometer tip across the edge of the cut film. Films with thicknesses ranging from 0.5 to 5.3 μm were used for these studies.

The electrode disks were mounted in a commercially-available PARC Teflon electrode holder (Model K0105); the exposed electrode area was 1 cm^2 . A PARC 1000 mL three-electrode electrochemical cell (Model G0096) was used. A saturated calomel electrode (SCE) equipped with a Luggin capillary was used as the reference electrode; two graphite rods were used as the counter electrode. All potentials are reported vs. SCE. Sulfuric acid solutions ranging in concentration from 5 M to 10 mM were used as the supporting electrolyte. All experiments were conducted at room temperature. To investigate the role of dissolved O_2 in the corrosion-protection process the electrolyte

solutions were either air-saturated or purged with N₂ or oxygen (Strate Welding Co., 99.5 %) during the electrochemical experiment.

Electrochemical Methods

A variety of electrochemical methods, including measurements of the open-circuit potential and the scanning reference electrode technique (SRET) were used to elucidate the mechanism by which PANI films passivate stainless steel surfaces in H₂SO₄ solution.

Open circuit potential measurements (OCP) and scanning reference electrode technique (SRET)

Measurements of the open-circuit potential as a function of time of exposure to the sulfuric acid solution were accomplished using a PARC 273A potentiostat/galvanostat controlled with PARC corrosion analysis software SoftCorr III. Cyclic voltammetric experiments were done using a PARC 263 potentiostat/galvanostat controlled with PARC Research Electrochemistry Software Model 270/250. The scanning reference electrode technique (SRET, EG&G PARC SRET Model SVP 100) was used to study passivation of pinholes in the PANI films. SRET is a microscopic technique used to identify and quantify regions of localized electrochemical activity on a sample surface immersed in an electrolyte solution. Briefly, SRET utilizes a fine platinum tip (2 to 5 μm diameter) that is positioned above the sample surface and scanned over the surface using a x-y scanning head (219). In addition to this x-y scanning a piezo-ceramic device is used to vibrate the probe tip at 80 Hz in the z direction. The amplitude of this vibration was 40 μm .

When a region of localized electrochemical activity (e.g., corrosion) is encountered, the ionic current flow produces a potential gradient in the solution above this region. This means that the potential at the high and low points of the tip oscillation will be different. The magnitude of this potential difference is a measure of the local current

density, and the sign of the potential difference indicates whether the local current is cathodic or anodic. With the PARC system, a negative potential difference corresponds to an anodic current. In order to maximize the potential difference, relatively low concentrations of electrolyte (≤ 0.01 M) are used. The spatial resolution of the technique is 5 to 10 μm (219)

Prior to measurements on the PANI films, the SRET probe tip was tested using the “point in space method” recommended by the manufacturer (219). This entails measuring the potential difference above a 0.2 mm-diameter Au electrode at which an anodic current of 10 μA is applied. The electrolyte was 10 mM H_2SO_4 . If the tip is functioning properly, a narrow bell-shape curve of potential difference vs. distance across the electrode surface is obtained. The EG&G PARC potentiostat/galvanostat Model 273A was used to apply the current.

The SRET has a triangular sample stage with a 3.2 cm-diameter hole into which the sample is mounted. SRET samples were prepared by embedding the stainless steel disks in epoxy resin (Buehler, epo-kwick resin #20-8129) using a 3.2 cm-diameter Buehler (#20-8180) plastic cold mounting mold. After embedding, epoxy residue was removed from the metal surface using 600 grit SiC paper. The metal was polished until a shiny surface finish was obtained, and the sample was rinsed and dried as described above. The PANI film was then applied and after solvent evaporation, an approximately 1 mm-diameter hole was punched through the film to expose the underlying metal. This was accomplished using a nail with an approximately 1 mm-diameter tip. The nail was punched through the film by tapping with a hammer. The sample was then placed in the sample holder and levelled using the adjustment screws on the sample stage.

The probe was then levelled and positioned above the sample near the hole. While observing through a magnifying glass, the oscillating tip was slowly lowered and positioned as close as possible, visually, to the specimen surface. The sulfuric acid solution was then added to the tank ($V=3.5$ L), and the phase angle (the difference in phase between the reference AC signal and the phase of the output signal) was adjusted as per the manufacturer's recommendation (219, 220). Finally, in analogy to the procedure of Powell et al. the probe was very slowly lowered (in increments of ~ 6 μm) while monitoring the output signal until contact was made with the PANI film (output signal = 100 %) (221). The tip was then retracted from the film to a height where the output signal was ~ 50 % (220). Data were obtained with the probe oscillating at this height. Images of the SRET system may be found on the PerkinElmer Ins. webpage (<http://www.par-online.com/products/localframe.html>).

Scanning electron microscopy (SEM), auger electron spectroscopy (AES), and UV/visible spectroscopy

SEM images of the PANI films were obtained on a JOEL JSM Model 6400 microscope. Auger spectroscopy was used to determine the atomic composition of the stainless steel surfaces and to obtain depth-profile information. Two types of samples were investigated. The first consisted of surfaces that had been coated with PANI and then exposed to H_2SO_4 solution. After exposure the PANI film was removed by applying and then removing a piece of Scotch tape, and the underlying stainless steel surface was analyzed. In addition, bare stainless steel electrodes that had been exposed to H_2SO_4 solution were analyzed. An AES Perkin-Elmer PHI 660 scanning Auger multiprobe instrument was used. Depth profile data were obtained by using an Ar-ion gun to remove successive layers of material. The total sputtering time was 10 min. UV/Visible spectra

were obtained for PANI films coated onto glass microscope slides. Both the emeraldine base and salt forms of the polymer were investigated. A Hitachi U-3501 spectrophotometer was used.

Results and Discussion

Characterization of the Polyaniline Films

Figure 5-1 shows UV/visible spectra for films of the emeraldine-base and emeraldine-salt forms of PANI. These spectra show the characteristic absorption bands for these materials (207, 222, 223). Figure 5-2 shows a cyclic voltammogram for a 0.5- μm -thick PANI film in 1 M H_2SO_4 . This voltammogram shows the two reversible redox waves characteristic of PANI (211). Starting at the most negative potentials in Figure 5-2, the first oxidation wave (1) corresponds to the oxidation of the leucoemeraldine salt to the emeraldine salt. The return wave (1') is the corresponding reduction. The second wave (2) corresponds to the oxidation of the emeraldine salt to the fully oxidized pernigraniline form of PANI. The return wave (2') is the corresponding reduction. Figure 5-3 shows a SEM image of a 0.5- μm thick as-prepared emeraldine-base film. Microscopic cracks are observed across the face of the film. As will be discussed below, for thin films these cracks allow electrolyte solution to access the underlying stainless steel surface.

Open-Circuit Potential Measurements

When a bare (no PANI film) stainless steel electrode surface is exposed to 1M H_2SO_4 solution, the open circuit potential (OCP) is initially negative of -500 mV but climbs to a steady-state value of $\sim$ -470 mV over a period of about 24 hours (Figure 5-4). Vigorous gas evolution is observed indicating, as expected, the rapid corrosion of the metal surface in this highly acidic environment.

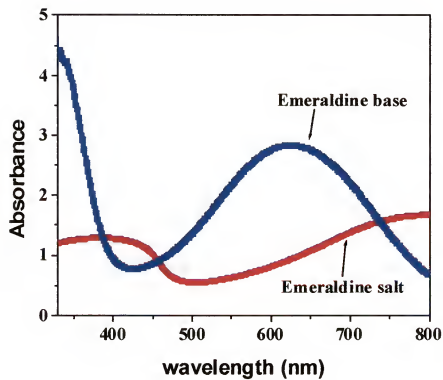


Figure 4-1. UV/visible spectra for the emeraldine-salt and emeraldine-base forms of PANI.

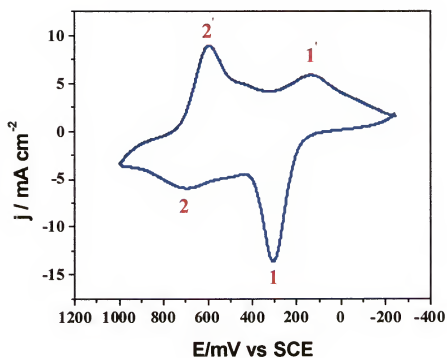


Figure 4-2. Cyclic voltammogram for a stainless steel electrode coated with a $0.5 \mu\text{m}$ thick PANI film in $1 \text{ M H}_2\text{SO}_4$.

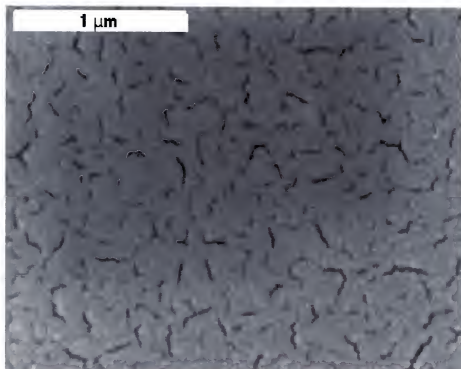


Figure 4-3. SEM image of a 0.5 μm -thick PANI film covering a stainless steel electrode surface.

In contrast, when a PANI-coated film is immersed in 1 M H_2SO_4 solution, the OCP is initially positive of 400 mV, dips negatively and then recovers to values well positive of 300 mV (Figure 5-5). The OCP stays at such very positive (relative to the bare electrode) values for periods in excess of one month. Furthermore, no gas evolution is observed. These results clearly show that the underlying stainless-steel surface is passivated by the PANI coating. Note, in particular that in contrast to the bare surface, the OCP of the PANI-coated surface is maintained in the passive region for stainless steel (225).

Such highly-positive OCP values for PANI-coated iron and stainless steel surfaces in acidic solutions have been observed previously (212, 214, 215, 225); however, the dip and recovery in the OCP has not been previously reported. The magnitude of the dip in

the OCP and the timescale of the recovery process depend on the thickness of the PANI film. Thinner films show a more dramatic dip and a slower recovery to more positive potentials (Figure 5-5). The dip and recovery process also depends on the concentration of the H_2SO_4 . More highly concentrated solutions cause the potential to dip more negatively and recover more slowly (Figure 5-6).

The SEM image in Figure 5-3 shows that there are cracks in the PANI film. For the thinnest films these cracks expose the underlying metal to the H_2SO_4 solution. This results in localized corrosion of the metal surface, which as will be discussed in detail below, causes partial reduction of the PANI film and the concomitant dip in the OCP. This is less of a problem for the thicker films, and as a result, the dip in the OCP is not as dramatic (Figure 5-5).

This interpretation is supported by the work of Ahmad and MacDiarmid who intentionally scratched PANI films on stainless steel surfaces and found that upon exposure to H_2SO_4 the OCP initially decreased but then returned to more positive values (226). We have obtained analogous results from scratched PANI films.

The mechanism by which the OCP recovers after the dip was investigated by studying the effect of dissolved O_2 on the rate of this process. The recovery of the OCP to more positive values is fastest when O_2 is bubbled through the H_2SO_4 solution and very slow when the solution is purged with N_2 (Figure 5-7). There are two possible explanations for this observation. The first is that the rate of recovery of the OCP corresponds to the rate of formation of a passivating oxide film over the regions of the steel that have corroded as a result of exposure to H_2SO_4 .

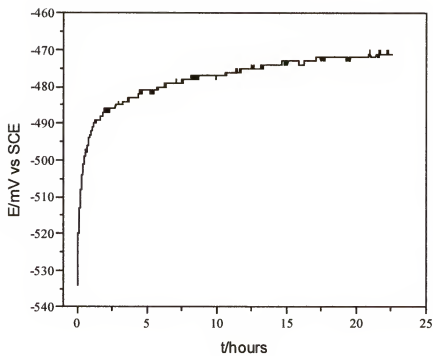


Figure 4-4. Open circuit potential vs. time data for an uncoated stainless steel electrode in 1 M H_2SO_4 .

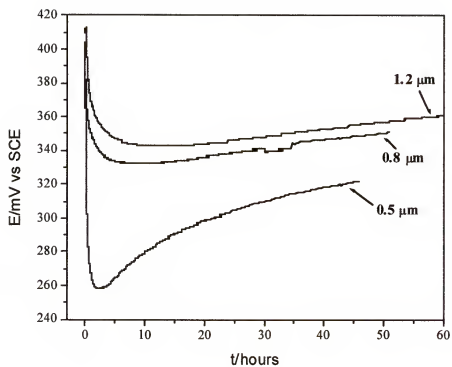


Figure 4-5. Open circuit potential vs. time data for stainless steel electrodes coated with PANI films of the indicated thickness, 1 M H_2SO_4 .

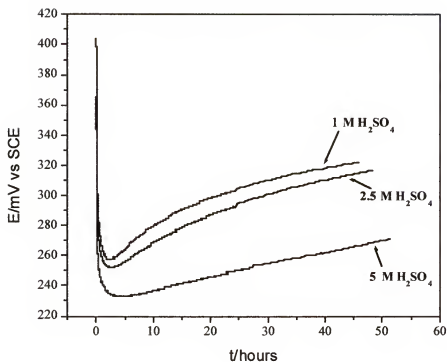


Figure 4-6. Open circuit potential vs. time data for stainless steel electrodes coated with 0.5 μm thick PANI films in H_2SO_4 solutions having the indicated concentration.

While numerous prior investigations have shown that oxide film does form and that formation of this film is critical to passivation of the steel surface, the source of oxygen for this film is water and not dissolved O_2 (227, 228). Therefore, if the rate of recovery of the OCP corresponds to the rate of oxide formation, a dependence on the concentration of dissolved O_2 in the solution (Figure 5-7) would not be expected.

The alternative explanation is that the electrons liberated by corrosion of the underlying steel go to reduction of the PANI film. The Nernst equation would, of course, predict that this reduction process would cause the potential of the film to drop. In this case, the recovery of OCP would correspond to the reoxidation of the PANI film by dissolved O_2 . The results in Figure 5-7 are in agreement with this model in that the rate of recovery of the OCP is strongly dependent on concentration of dissolved O_2 in the solution. In the N_2 -purged solution only traces of adventitious O_2 are present and this is why the reoxidation process occurs so slowly.

This model - that corrosion results in reduction of the PANI film and O_2 reoxidizes the film - has been proposed previously (140, 229). However, these authors suggest that the presence of O_2 is critical to the passivation process, because the O_2 reduced at the PANI surface is used to form the passivating oxide film. As noted above, this is in disagreement with accepted models for oxide formation at steel surfaces, which assume that H_2O is the source of oxygen for the oxide (227, 228). In addition, this is in disagreement with our experimental data because we obtain a passivated electrode (i.e., OCP in the passive region) when N_2 is purged through the solution (224).

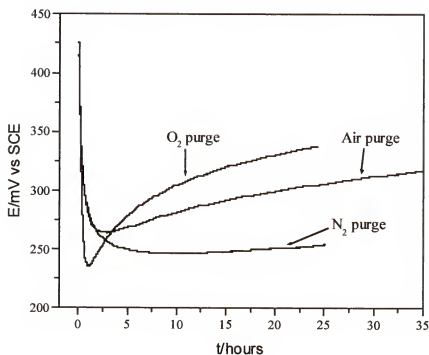


Figure 4-7. Open circuit potential vs. time data for stainless steel electrodes coated with $0.5\text{ }\mu\text{m}$ -thick PANI films in $1\text{ M H}_2\text{SO}_4$ solutions that were purged with O_2 , air, or N_2 .

If oxide formed from O_2 reduced at the PANI surface were essential (as proposed in the prior studies (140, 229)), passivation would be difficult to achieve in the N_2 -purged solution. It is important to note that these prior investigations did not include studies of the effect of dissolved O_2 on passivation.

The correct mechanism by which oxidized conductive polymers passivate active metal surfaces was, in fact, elucidated by Deng et al. in 1989 (218). According to these authors, the high positive redox potential of the oxidized conductive polymer film maintains the potential of the metal surface in the passive region where the rate of corrosion is very low. In essence the Fermi level of the underlying metal is shifted to more positive values by being in ohmic contact with the doped conductive polymer. This conclusion is supported by Deng et al.'s observation that the conductive polymer film does not have to be coated onto (or in physical contact with) the metal surface itself (231). In their studies the film was coated onto a glassy carbon electrode which was connected via a wire to the metal surface to be passivated.

Our studies (and the work of Ahmad and MacDiarmid (226) and Kinlen et al. (140)) confirm that not all of the metal surface must be coated in order to obtain passivation. We have observed blistering of the PANI film after long exposure times to the sulfuric acid solution (Figure 5-8). These blisters expose bare metal to the solution, yet the surface remains passivated. To emphasize this point we intentionally coated only half of an electrode surface with PANI and left the other half bare. Figure 5-9 shows the OCP vs. time plot for this half-coated sample upon immersion into 1 M H_2SO_4 . The typical dip and recovery of the OCP is observed, and the potential is in the passive region.

Figure 5-10 shows results of another experiment that illustrates the point that the metal surface does not need to be completely covered by the PANI film. A stainless steel surface was initially completely coated with a 0.8 μm -thick PANI film, and the electrode was immersed into 1 M H_2SO_4 . The typical dip and recovery of the OCP is observed (Figure 5-10). After approximately 44 hours, the electrode was taken out of the solution and half of the PANI film was removed by applying and removing a piece of Scotch[®] tape. The electrode was then reimmersed into the H_2SO_4 solution. As shown in Figure 10, this has no measurable effect on the OCP, again clearly showing that not all of the metal surface needs to be coated in order to achieve passivation.

At ~46 hours the electrode was again removed from the H_2SO_4 solution, and the PANI film remaining on the surface was scratched with the tip of a pair of forceps. When the electrode was reimmersed, the OCP dipped because localized corrosion of the exposed bare metal causes partial reduction of the PANI film. The OCP then recovers as before due to reoxidation of the film by dissolved O_2 . Finally after ~69 hours the electrode was again removed and a scratch was made on the portion of the metal not covered by the PANI film. When the electrode was reimmersed another dip and recovery was observed.

Auger Spectroscopy

Figure 5-11 shows the Auger spectrum for a bare stainless steel sample that had been exposed to 1M H_2SO_4 for 48 hours, and Figure 5-12 shows the corresponding depth profile for this sample.



Figure 4-8. SEM image a 0.8 μm -thick PANI film coated onto a stainless-steel electrode surface after 20 days of exposure to 1 M H_2SO_4 .

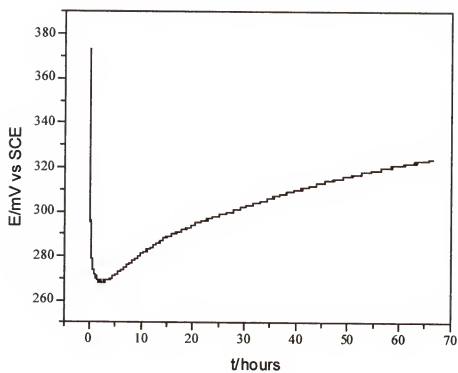


Figure 4-9. Open circuit potential vs. time data in 1 M H_2SO_4 for a stainless steel electrode whose surface was only half coated with a PANI film.

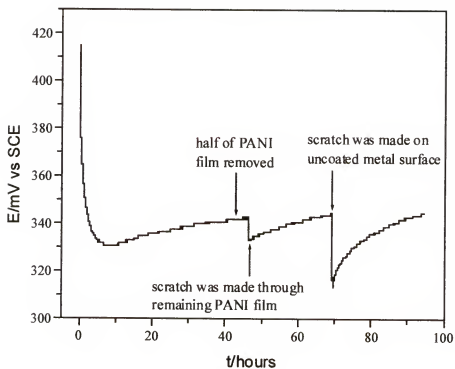


Figure 4-10. Effects of removing half of the PANI film and of scratching on the open circuit potential vs. time data for a stainless steel electrode that was coated with a 0.8 μm -thick PANI film, 1 M H_2SO_4 .

The depth profile shows that the surface region is depleted in Fe and enriched in O, relative to the bulk metal, observed at long (>5 min) sputtering times. These data indicate that the surface is coated with a thick (compared to the PANI-coated samples, *vide infra*) oxide film. In addition, there is no Cr present at the stainless-steel surface, and a sputtering time of >1 min is required to access Cr.

Figures 5-13, 5-14 and 5-15 show depth profiles for PANI-coated samples that had been exposed to 1M H₂SO₄ for various lengths of time. After the desired exposure time, the PANI film was removed with Scotch tape, and the Auger data were obtained from the underlying stainless steel surface. After 5 min of exposure to the sulfuric acid solution (Figure 5-13), a thin (relative to the uncoated surface) oxide layer is observed, and again there is no Cr at the surface. After 48 hours of exposure to the solution (Figure 5-14), the oxide layer is still thin, but now the surface is enriched in Cr. After 7 days of exposure (Figure 5-15), the oxide layer thickens significantly; however, it is still thinner than the oxide film on the bare surface that had been exposed to the sulfuric acid solution for only 48 hours. In addition, the surface for the 7-day sample is further enriched in Cr (Figure 5-15).

Kirchheim et al. studied the fundamentals of corrosion of iron-chromium alloys in 1M H₂SO₄ (230). In analogy to our results, they found that Cr is enriched at the surface when the potential of the electrode is held in the passive region. In contrast, at potentials in the active region, the Cr content at the stainless steel surface was low. The enrichment of Cr in the passive film of Fe-Cr alloys is due to both the lower mobility of Cr in the film and the preferential dissolution of iron into the electrolyte (231).

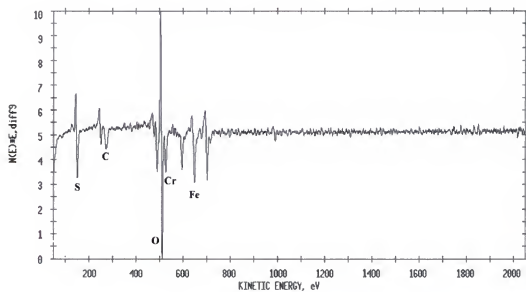


Figure 4-11. Auger spectrum for an uncoated stainless steel electrode that had been exposed to 1 M H_2SO_4 for 48 hours.

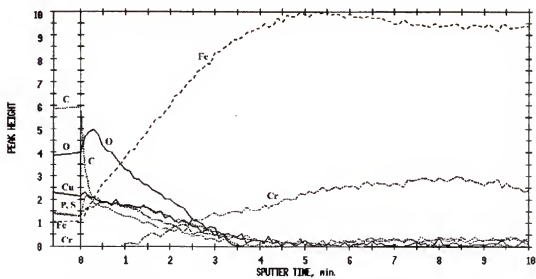


Figure 4-12. Auger depth profile data for the electrode in Figure 4-11.

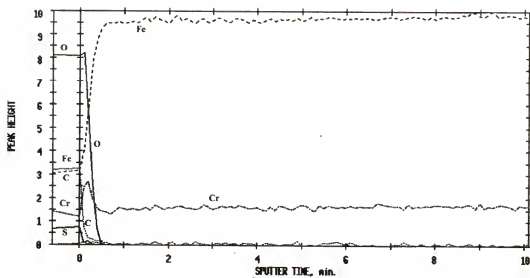


Figure 4-13. Auger depth profile data for a stainless steel electrode that had been coated with a 0.8 μm -thick PANI film and exposed to 1 M H_2SO_4 for 5 minutes.

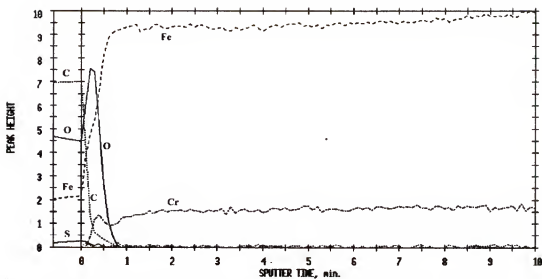


Figure 4-14. Data as per Figure 4-13 but after 48 hour exposure to 1 M H₂SO₄.

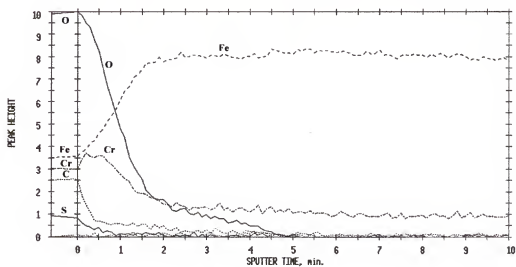


Figure 4-15. Data as per Figure 4-13 but after 7 day exposure to 1 M H₂SO₄.

These results on Cr enrichment support the conclusion that the PANI film passivates the underlying surface by simply maintain the potential of the electrode in the passive region.

Scanning Reference Electrode Technique (SRET)

SRET has been used to study weld-metal corrosion (231), localized corrosion of stainless steels (232, 233), carbon steel (141), and galvanized steels (221, 234), as well as pit initialization (235). Its principle attractive feature is that it can map out regions of localized corrosion while the sample is still immersed within the test solution. We have used SRET here to study passivation of the stainless steel in pinholes intentionally punched through the overlying PANI film.

Figure 5-16 shows SRET scans over a single pinhole (1 mm diameter) in a 2 μm -thick PANI film covering a stainless steel sample immersed in 0.01M H_2SO_4 . At short times after immersion in the H_2SO_4 solution corrosion of the stainless steel in the pinhole region is observed, as indicated by the region of negative potential gradient (anodic currents, dark blue color) above the pinhole (Figures 16A and B). At longer times this region of localized corrosion “heals,” as indicated by the decreasing potential gradients above the pinhole (Figures 5-16C and 5-16D). After ~ 4 hours the local corrosion has stopped, as indicated by the uniform and less than 20 μV (within the noise level) variation of the potential across the surface. Similar results were observed by Kinlen et al. for PANI-coated carbon-steel samples immersed in tap water (140). We were interested in learning whether this “self-healing” phenomenon would be observed for the much more aggressive H_2SO_4 environment studied here. Finally, these studies further

confirm the conclusion that not all of the steel surface must be coated with PANI to observe passivation.

Conclusions

The objective of this work was to elucidate the mechanism by which PANI films passivate stainless steel surfaces in highly corrosive H_2SO_4 solution. We have found that passivation is achieved because the PANI film holds the potential of the stainless steel electrode in the passive region. One of the most interesting results in this regard comes from the Auger studies of surface Cr. In analogy to the case of a bare stainless steel surface whose potential is potentiostatically maintained in the passive region (244), the PANI-coated surfaces are enriched in Cr after exposure to H_2SO_4 solution. This model for corrosion inhibition by PANI is also supported by our various studies on partially-coated electrode surfaces. We have also used SRET to investigate passivation of corrosion at pinholes intentionally cut through the PANI film. We have found that even in this highly corrosive medium, the PANI film can passivate the exposed stainless steel in the pinhole region.

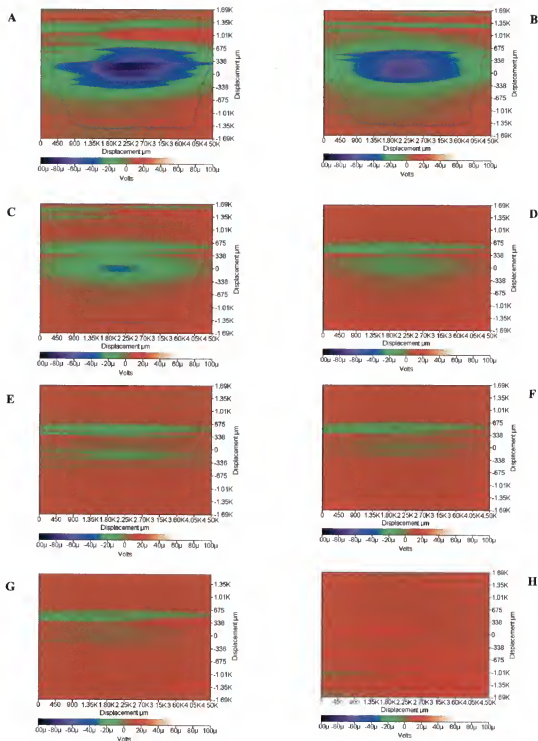


Figure 4-16. SRET images for a stainless steel electrode coated with a 2 μm -thick PANI film containing a 1 mm-diameter pinhole. Images obtained while the sample was immersed in 0.01 M H_2SO_4 . Immersion times were: A. 1 min. B. 10 min. C. 30 min. D. 60 min. E. 3 hr. F. 4 hr. G. 5 hr. H. 12 hr.

CHAPTER 5 CONCLUSIONS

Chapter 2 shows that single-stranded homo-oligonucleotides can be driven through the nanopore via the electrokinetic transport processes of electrophoresis and diffusion. J_{diff} decreases with increasing DNA base number indicating that these membranes act as size-based ultrafiltration membranes. The total flux with the current on is in all cases greater than the flux with the current off. The high ionic charge of the DNA insures that the contribution of electrophoretic transport is high. The EOF rate is dependent on the charge density on the pore walls. In our case the charge density of the pore walls is low which contributes to the lack of a significant EOF term. Thus, the dominant electrokinetic transport phenomena for DNA in these membranes is electrophoresis. The E_{ek} values show that electrophoresis dramatically enhances DNA transport, especially for the larger DNA chains. Transmission electron microscopy results showed that pores in polycarbonate membranes are cigar-shaped. Thus, they do not have a constant diameter through out the membrane thickness.

Chapter 3 describes a sol-gel template synthesis process was used to prepare silica nano-test-tubes within the pores of alumina template membranes. We have shown that these test tubes are closed on one end, open on the other, and hollow. The length of these test tubes can be controlled at will by varying the thickness of the template membrane used. We have also shown that that silica nanotubes can be chemically and biochemically functionalized using silane chemistry with commercially available reagents. The inner surfaces of the silica nano-test tubes were functionalized with green

fluorescent silane and the outer surfaces of the silica nano test tubes were tagged with antibodies that promote uptake by endothelial cells. The final challenge to be addressed if these test tubes are to be used for biomolecule delivery is to develop procedures for capping and uncapping the open mouth.

Chapter 4 shows the mechanism by which PANI films passivate stainless steel surfaces in highly corrosive H_2SO_4 solution. We have found that passivation is achieved because the PANI film holds the potential of the stainless steel electrode in the passive region. One of the most interesting results in this regard comes from the Auger studies of surface Cr. In analogy to the case of a bare stainless steel surface whose potential is potentiostatically maintained in the passive region (244), the PANI-coated surfaces are enriched in Cr after exposure to H_2SO_4 solution. This model for corrosion inhibition by PANI is also supported by our various studies on partially-coated electrode surfaces. We have also used SRET to investigate passivation of corrosion at pinholes intentionally cut through the PANI film. We have found that even in this highly corrosive medium, the PANI film can passivate the exposed stainless steel in the pinhole region.

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BIOGRAPHICAL SKETCH

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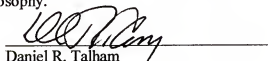
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


John R. Reynolds, Chairman
Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


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Weihong Tan
Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

A handwritten signature in dark ink, appearing to read "John G. Harris", is written over a horizontal line.

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This dissertation was submitted to the Graduate Faculty of the Department of Chemistry in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 2003

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